EFFECTS OF *Tithonia diversifolia* EXTRACT AND *Trichoderma asperellum* ON *Botrytis cinerea* GROWTH YIELD AND QUALITY OF STRAWBERRY (*Fragaria ananassa var duch)*.

WANJUKIA JULIA WAMBUI

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Horticulture of Egerton University

EGERTON UNIVERSITY

APRIL, 2021

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this University or any other for the award of degree

Signature ___ __Date____21/4/2021_____

Wanjukia Julia Wambui

KM14/11692/16

Recommendation

This thesis has been submitted with our approval as University supervisors.

Signature _____ Date___21.4.2020_____

Dr. Robert Morwani Gesimba, Ph.D.

Department of Crops, Horticulture and Soils

Egerton University

Signature _____ _____ Date_____21/4/2021_____

Dr. Joseph Ngwela Wolukau, Ph.D.

Department of Crops, Horticulture and Soils

Egerton University

COPYRIGHT

DEDICATION

I dedicate this thesis to my parents, sisters, husband, daughter, and my son whose encouragement and prayers have been a source of inspiration and strength in this academic journey.

ACKNOWLEDGEMENTS

I thank the Almighty God for his grace and mercy for granting me with courage, energy, guidance, and good health throughout my study period. I am grateful for the financial support from RUFORUM through TAGDev PROGRAM at Egerton University which has made my study successful. I am also grateful for Egerton University for granting me the opportunity to pursue my master degree. My profound gratitude goes to my supervisors, Dr. Robert Gesimba and Dr. Joe Wolukau for their tireless guidance and constructive comments throughout the study. Special thanks go to the staff of the Department of Crops, Horticulture, and Soils of Egerton University for the moral support they accorded me during my studies. I am also grateful to Horticulture the Principal Technologists, Horticulture section Mr. Jacob Ochieng and for their technical support and advice. I am also grateful to my family, colleagues, Grace Kamau, Francis Kimani, Jane Njeri, Dennis Kimani, Shantel Wangari, Jayden Ndirangu and all those who might have contributed to this work in one way or the other.

ABSTRACT

Strawberry production and consumption is experiencing significant growth in Kenya and globally due to its increasing use in the food processing industry. The fungus *Botrytis cinerea* is a major pathogen causing Grey mould in strawberry and can cause a significant economic loss of fruits both in the field and in storage. The pathogen is controlled using synthetic fungicides, but new strains develop resistance very fast making it difficult to control. Therefore, there is a need for alternative methods of control using biological agents that are environmentally friendly and not harmful to human beings. This study was designed to contribute towards improved strawberry production in Kenya through reduced *Botrytis cinerea* incidence using *Tithonia diversifolia* (*T. diversifolia*) extracts and *Trichoderma asperellum* (*T. asperellum*). Two experiments were conducted at the Horticulture Research Field, Egerton University. The factorial experiments were laid out in a Randomized Complete Block Design with three replications. There were two factors i.e *T. diversifolia* and *T. asperellum. Tithonia diversifolia* was applied at four concentrations (0mL/L, 250mL/L, 500mL/L, and 750mL/L) while *T. asperellum* was applied at three levels (0mL, 40mL and 80mL per 20 liters of water). Data were collected on; growth, *Botrytis cinerea* incidence and severity, fruit yield, and fruit quality. *T. diversifolia* and *T. asperellum* influenced growth and yield of strawberry fruits. A Combination of *T. diversifolia* at 750mL and *T. asperellum* at 80mL produced the highest number of leaves (58.83) while control produced the least (43.63). *T. diversifolia* and *T. asperellum* influenced *Botrytis cinerea* incidence and severity in strawberry fruits. There was a decrease in disease incidence at *T. asperellum* 80mL and *T. diversifolia* 750mL (16.49%) compared to control (85.27%). A combination of *T. asperellum* and *T.diversifolia* reduced disease severity by recording the lowest percent (12.07%) compared to control (89.04%). Different concentrations of *T. diversifolia and T asperellum* had significant $p \leq 0.05$ effects on strawberry fruit yield where *T. diversiofolia* at 750 mL and *T. asperellum* at 80mL produced the highest yields (199.5g per plant) compared to control(53.9 g per plant). Total soluble solids was highest in fruits treated with *T. diversiofolia* at 750 mL and *T. asperellum* at 80mL (13.28 brix) compared to control (7.97 brix). Ascorbic acid content was highest in fruits treated with *T. diversiofolia* at 750 mL and *T. asperellum* at 80mL (55.12mg/100g) compared to control (38.75mg/100mg). Based on the results, *T. diversifolia* and *T. asperellum* significantly ($p \leq 0.05$) influenced disease incidence and severity, growth and Fruit yield of strawberry. Consequently, *T. diversifolia* at 750mL and *T. asperellum* at 80mL are recommended for use in strawberry production.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

FAO	Food and Agriculture Organization
HCD	Horticulture Crops Directorate
PDA	Potato Dextrose Agar
USA	United States of America
DAP	Days After Planting

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Fruits constitute a significant part of human nutrition and are highly recommended for a health vitamin-rich diet. World wide, more than 675 million metric tons of fruit are produced every year. Fruit production is one of the major sources of domestic income in Kenya. In 2014 fruits contributed KES 51.4 billion accounting for 26 percent of the domestic value of horticultural produce (HCD, 2014).

Strawberry (*Fragaria ananassa var duch*) is a small soft fruit crop, which belongs to the family Rosacea and genus Fragaria. The modern cultivated berry *Fragaria ananassa* is a hybrid between *F. virginia* (meadow strawberry) and *F. chiloensis* (Chilean strawberry) (Martin & Tepe, 2014). Strawberry is one of the most significant temperate small soft fruit crops throughout the world. The first reference to strawberries comes from ancient Rome, but the fruits were likely collected from the wild for curative purposes and as a source of nourishment long before recorded history (Martin & Tepe, 2014). Commercial production of strawberry crops through the 19^{th} and 20^{th} centuries increased rapidly as strawberries became more popular. Tonnes of fresh fruit are consumed every year but there are also chances for the use of second-grade berries in fruit juice, dried, and processed products. There is also an increasing interest in the health benefits of strawberries, this factor aids to promote year round strawberry sales (Davis, 2015). The fleshy fruit of strawberry is classified as an aggregate fruit (Martin & Tepe, 2014). Strawberries are distinctive with highly desirable taste as a result of sugars and flavour fiber, potassium, and an excellent source of vitamins, potassium (Basu *et al.,* 2014). As compared to other berry fruits, strawberries contain a higher percentage of flavonoids, phenolics, and vitamin C (Hernandez *et al*., 2016).

Many European nations have been producing large volumes of greenhouse out of season strawberries. Canada, Spain, and the USA annually plant 9,700, 16,800, and 55,000 ha, respectively (Food and Agriculture Organization of the United Nations, 2008). In the US, Florida has the largest production hectarage, which in 2008 generated approximately 330 million USD (U.S. Department of Agriculture, 2008) (Table 1).

Table 1 .Worldwide distribution of strawberries 2008 to 2012 (Tons per year)

Position country	**Country**	**Production tons per year**				
		2008	2009	2010	2011	2012
1	USA	1148350	1270640	1294180	1312960	1366850
2	Mexico	207485	233041	226657	228900	360426
3	Turkey	261078	291996	299940	302416	353173
4	Spain	281240	266772	275355	262730	289900
5	Egypt	200254	242776	238432	240284	242297
6	S Korea	192296	203772	231803	171519	192140
7	Japan	190700	191400	190700	184700	185000
8	Russia	180000	185000	165000	184000	174000
9	Germany	150854	158563	156911	154418	155828
10	Poland	200723	198907	153410	166159	150151
	Total world production	**4130279**	**4596586**	**4352869**	**4328129**	**4516810**

Source: FAO (2012)

In Kenya Strawberry is mainly grown for the domestic market. The main varieties grown include Aiko, Douglas Chandler, Cambridge favourite among others (HCD, 2014). The demand for strawberry has been on the increase in recent years. In 2014, the area under production in Kenya was 100 ha, producing 1,487 MT with a value of KES 144 Million. The main strawberry producing counties include Taita Taveta, Kiambu, Meru, and Nakuru accounting for 41, 36, 14, and 8 percent respectively of the total value of strawberries produced in the country. The potential for increased production of strawberry is huge due to the ready market in the food processing industry. The major constraints to increased production of strawberry are pest and disease incidence, limited knowledge on appropriate agronomic practices among growers, lack of suitable day-neutral varieties, inadequate quality planting materials (HCD, 2014). Table 2 shows the strawberry production statistics in Kenya.

Table 2. Production of Strawberries by County 2012-2014 in Kenya

County	2012		2013		2014	
	Volume (MT)	Value (Million KES)	Volume (MT)	Value (Million KES)	Volume (MT)	Value (Million KES)
TaitaTaveta	423	46.1	526	58.0	59	22
Kiambu	460	47.0	392	57.1	52	17
Meru	-	-	-	-	34	11
Nakuru	-	-	-	-	10	3
Murang'a	0	0.2	0.2	1	0.6	
Kirinyaga	2	0.2	3	0.2	5	1.5
Total	**885**	**93.5**	**921**	**115.5**	**162**	**55**

Source: HCD (2014)

Botrytis cinerea is considered the main pathogen causing disease in strawberry (*Fragaria x ananassa)* production because the fungus can infect leaves, stems, flowers, and fruits at any stage of development (Guetsky *et al*., 2011). Infected organs become necrotic, with intense sporulation, and a gray color, which characterizes the disease (Costa *et al*., 2013). Although the fungus can infect all parts of the plant, the damage is more common during the production phase when the pathogen infects flowers (Costa *et al*., 2013). The disease can cause heavy fruit losses on strawberry plants before or after harvest and it is estimated that 50% fruit loss for untreated strawberries can occur (Petrasch *et al*., 2019).

Grey mould (*B. cinerea*) is also a major cause of postharvest losses of strawberry fruits during storage, transportation, or shipment. *Botrytis cinerea* infections largely limit the postharvest life of strawberry fruits. Control of *B. cinerea* on strawberries can be achieved with the frequent application of fungicides; however, the resistance of the pathogen to common fungicides is well known (Cosseboom *et al*., 2019; Konstantinou *et al*., 2015). Fungicide applications can also result in toxic residues on fruits (Kim *et al*., 2016). Fungicide impacts have made farmers look for an alternative method of disease control such as Biological agents.

Biological suppression of plant diseases has been promoted as a means of achieving improved and sustainable crop production systems that are less reliant on chemical inputs (Sylla *et al.*, 2015). The presence of fungal diseases in strawberry and its economic

consequences require many fungicides. Modern production of strawberry is striving to involve biocontrol of diseases and limited chemical usage. For long, trichoderma species have been known as biological agents for control of plant diseases. Biological control offers an environmentally friendly approach for managing plant disease and can be incorporated into cultural and physical control and limited chemical use for effective integrated disease management (Biljana & Jugoslav, 2012).

Trichoderma asperellum is a species of fungus in the family Hypocreaceae and has been used in different fields of production and protection in agriculture (Herrera-Para *et al*., 2017; Jacometti *et al*., 2010). No harmful side effects have been reported for *Trichoderma* spp. on human or animal health or the environment (Schuster & Schmoll, 2010). Many commercial strawberry growing companies are using this fungus to produce biological plant protection products or plant growth promoters. These microbial products work by producing toxic compounds with a direct antimicrobial activity against pathogens

The toxic compounds include hydrolysing enzymes such as proteases, chitinases, lipases, esterases glucanase which can degrade cell walls of the pathogens (Xiaoxue *et al.,* 2013). The Antimicrobial activities could be the result of several secondary metabolites such as peptaibols, terpenes, polyketides, gliotoxin, and gliovirin produced by fungi (Vinale *et al.,* 2012). Other metabolites include tricholin, harzianic acid, viridian, gliosoprnins, heptelidic acid, 6-pentyl-α-pyrone, and massoilactone, which inhibit phytopathogen fungi growth (Mukherjee *et al*., 2012). These beneficial microorganisms compete for available resources with plant pathogens starving them to death. They are nature-friendly hence used to overcome problems caused by conventional standard chemical methods of plant protection and are used in organic systems of food production (Kowalska, 2010).

Tithonia diversifolia is a species of flowering plant in the family Asteraceae that is commonly known as the Mexican sunflower. The plant has high nutrient content and also has antifungal properties hence has been used for control of various plant pathogens (USDA, 2015). Tithonia extracts have inhibitory effects on the growth of fungal pathogens. *Tithonia diversifolia* contains several compounds such as saponins, sesquiterpenes, and alkaloids which makes it active in inhibiting mycelia growth (John *et al*., 2013; Tona *et al*., 2014). It has been effectively used in controlling fungal diseases such as *Fusarium solani*, *Fusarium lateritiuma, Curvularialunata* (Ilondu *et al.,* 2014).

1.2 Statement of the Problem

Strawberry is grown worldwide and the area of cultivation is expanding. *Botrytis cinerea* (grey mould) is one of the most important pathogens in strawberry production causing significant pre- and post-harvest loss of fruits because the fungus can develop both in the field and during the postharvest chain. Grey mould is also a major cause of postharvest losses of strawberry fruits during storage, transportation or shipment, therefore, limits the postharvest life of strawberry. Fruit susceptibility to fungal disease increases as ripening progresses; hence, *B. cinerea* appears to promote susceptibility in unripe fruit by activating specific ripening-related processes. Besides hormones, increased oxidative reactions caused by the pathogen may influence ripening progression. Management of *Botrytis cinerea* is based principally on chemical control; however, using fungicides in controlling the *Botrytis cinerea* infection might be undesirable as a result of their toxic residues in addition to the continuous emergence of resistant variants/strains of the pathogen. Control of fungal pathogens is based on the use of agronomic practices and pesticides, but the widespread application of chemicals fills the agro-ecosystems with toxic compounds that affect the natural food chain balance. These residues are harmful to human beings, animals, and the environment. These chemicals are not readily affordable and this has led to the high cost of production leading to low income.

1.3 Objectives

1.3.1 General Objective

To contribute towards reduced *Botrytis cinerea* incidence, improved growth, fruit yield, and quality, of strawberry (*Fragaria ananassa* Var *duch)* in Kenya by using *Trichoderma asperellum* and *Tithonia diversifolia*, which are eco-friendly.

1.3.2 Specific Objectives

i) To determine the effects of different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperrellum* on growth and fruit yield of strawberry
ii) To determine the effects of different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* on *Botrytis cinerea* incidence and severity in strawberry
iii) To determine the effects of different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* on fruit quality of strawberry

1.4 Hypotheses

i) Different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* have no significant effect on the growth and yield of strawberry.
ii) Different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* have no significant effect on *Botrytis cinerea* incidence and severity in strawberry
iii) Different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* have no significant effect on the quality of strawberry.

1.5 Justification of the Study

In Kenya, strawberry is mainly grown for the domestic market. The demand for strawberry has been on the increase in recent years. In 2014, the area under production was 100 Ha, producing 1,311 MT with a value of KES 144 Million (HCD, 2014). Strawberry gardening is an excellent opportunity particularly for the youth in Kenya for self-employment and livelihood improvement. Strawberries are easy to grow, require minimal land, and thrive well in a wide range of temperatures ranging from mild to hot hence they can help in poverty alleviation and rural development in Kenya. Strawberries are rich sources of nutritive compounds including minerals, vitamins, fatty acids, fibres, and secondary metabolites, as polyphenols, which are the most diffused and interesting bioactive compounds present in this fruit (Giampieri *et al*., 2012).

The main class of strawberry polyphenols are flavonoids, followed by tannins, flavonols, and phenolic acids: all these compounds show huge biological potentialities in humans, from antioxidant capacity to anti-inflammatory, anti-hypertensive, and anti-proliferative abilities (Giampieri *et al.,* 2015). These properties help in the prevention and improvement of chronic diseases. *Botrytis cinerea* is one of the most economically important pathogens of strawberry, which causes a loss of 25% of strawberry fruit yield (Mertely, 2019). The disease is controlled using fungicides, which are harmful to both human beings and animals. Continuous use of fungicides pollutes the environment hence it is important to look for cost -effective control measures that have no harmful effect on humans, animal health, and environment. *Trichoderma asperellum* and *Tithonia diversifolia* if proven effective will reduce the *Botrytis cinerea* leading to increased yield and shelf life hence more income to farmers.

CHAPTER TWO
LITERATURE REVIEW

2.1 Botany of Strawberry

The strawberry crop (*Fragaria x ananassa*) is considered an important commodity worldwide (Banaeian *et al.*, 2011). Strawberry is a small herbaceous perennial plant, which can be grown as an annual or perennial crop under commercial cultivation. Strawberry plants consist of a crown (shortened stem) from which leaves, roots, flowers, and runners grow. The crown is very important for the survival of the plant due to its ability to store reserves for plant growth after chilling dormancy. Strawberry plants can consist of a single crown or can exist as double or multiple crowned plants depending on age and stage of development. Freshly planted runners normally consist of a single crown, while 2 to 3 year old plants develop multiple crowns consisting of both auxiliary and branch crowns. Branch crowns do not have their root system but allow the plant to expand in width (Rubinstein, 2015).

Strawberry leaves are trifoliate and normally last for about 1 to 3 months. During growth, the crown elongates and produces new leaves. The buds in the axils can give rise to runners for the production of new plants, in some instances; strawberry can form secondary crowns as the plant matures (Martin & Tepe, 2014). Strawberry flowers are produced on a modified stem, which is terminated by the primary flower. Further stems can rise from the main stem to produce secondary flowers from which tertiary flowers arise (Rubinstein, 2015) According to Hancock (2013), following the primary flower there are typically two secondary, four tertiaries, and eight quaternaries. This results in a highly branched flower stem or truss structure. These flowers open in succession. Therefore, if the environmental conditions are ideal and pollination occurs a successive fruit harvest can be obtained from each flower truss (Davis, 2015).

The strawberry is an aggregate fruit composed of numerous ovaries each with a single ovule. The resulting seeds are called achene's and are the true fruits of strawberry. The embryo consists of two large semi -elliptical cotyledons, which contain protein and fat but no starch. The receptacle is composed of an epidermal layer, cortex, and pith. The latter two layers are separated by vascular bundles that supply nutrients to the developing embryos (Hancock, 2013). The development of the fruit depends on the maintenance of a hormonal balance during achene maturation. Any interruption of that balance, incomplete fertilization, or death of the achene's from any on several of pathogenic or non-pathogenic causes (for

example. infertile pollen frost injury insect attack or pathogenic fungal attack of flowers parts) results in malformed fruit (Davis, 2015).

Strawberry grows fast (two to three months) and is highly influenced by environmental conditions, such as light, salinity, water quality, temperature, and nutrients (Li *et al*., 2010). Due to its fast development, the crop needs sufficient absorption of macronutrients in order to meet the photosynthetic demand and adequate fruit growth (Li *et al*., 2010).

Figure 1.Strawberry fruits

2.2 Nutritive Value and Health Benefits of Strawberry

Strawberries are nourishing fruits with putative health benefits, because of their rich nutrient content, unique colour, flavour, and taste (Mahmood *et al.*, 2012). They are widely eaten fresh or consumed in processed forms combined with dairy products. Fruits and vegetables are the main sources of minerals and vitamins, which are very important for human health (Giampieri *et al*., 2012). Epidemiological researchers have shown that people who consume diets rich in vegetables and fruits have fewer incidences of chronic diseases such as cardiovascular, neurological disorder, cancer, infections, and diabetes (Vauzour *et al*., 2010). Strawberries are a natural source of micronutrients such as minerals, folates, phytonutrients, and vitamin C (Basu *et al*., 2014) .Strawberries, are mainly available in fresh or frozen forms, however, in in many countries they are also commercially available as processed products, such as nectar, jams, puree, and juices. In some cases, they are also used as the main berry ingredient in cuisine (Klopotek *et al*., 2015). Nutrition is not the only health

benefits in strawberry, other benefits includes ant proliferative, antihypertensive, anti-inflammatory, anti-hyperlipidaemia and antioxidant effects.

2.3 Environmental Requirement of Strawberry

The plant thrives on loam, well-drained soils with a pH of 5.5–6.5. Temperature requirements are in the range of 10 to 30°C and an average rainfall of 900 to 1200 mm (Kasperbauer, 2010). However, in places where this is not attainable then 25mm per week or irrigation is necessary (Kasperbauer, 2010). Though production can take place in different areas, the ideal altitude is from 1500m-2200m above sea level (Martínez-Ferri, 2016)

2.4 Constrains of Growing Strawberries

Chandler variety is the main variety grown in Kenya. It originated from the United States of America (FAO STAT, 2014). Strawberries from Kenya are mainly exported to countries such as Germany, Holland, United Kingdom, Middle East, Saudi Arabia, and France. There are relatively few Kenyan based studies on the production of strawberries, although reports show production has been declining from 2014 to 2016 (FAOSTAT, 2018), partly due to water scarcity, pests, disease, high cost of inorganic fertilizers and lack of knowledge on appropriate planting materials and management practices.

2.5 Grey Mould caused by *Botrytis cinerea*

Botrytis cinerea is a typical high-risk neurotropic parasitic fungus, responsible for grey mould, one of the most important plant diseases in strawberry production, which causes losses on plants and fruits. The fungus has a broad host range and can infect over 1000 plant species (Fillinger & Elad, 2016). The disease can cause extensive fruit losses on strawberry plants before or after harvest worldwide and it is estimated that it can cause yield losses of up to 25% for untreated strawberries (Williamson *et al*., 2007). Grey mould is also a major cause of postharvest losses of strawberry fruits during storage and transportation. In strawberry, the fungus can attack flowers, fruits, and leaves as well (Carisse, 2016). Infection in the flower, remain quiescent until fruits mature, and then develop abundantly, causing fruit decay accompanied by profuse sporulation of the pathogen (Haidar *et al*., 2016).

Botrytis cinerea is a high-risk plant pathogen due to its high genetic variability, short life cycle, and prolific reproduction (Angelini *et al*., 2016; Elad *et al*., 2016). These characteristics also favour the development of fungicide resistance, which reduces the efficacy of specific synthetic fungicides. Resistance phenomena by Botrytis *cinerea* have been analysed in numerous studies on different crops (Fillinger & Walker, 2016; Walker *et al*., 2013).Control of *Botrytis cinerea* on strawberries can be achieved with the frequent

application of fungicide. However, resistance of the pathogen to common fungicides is well known (Hahn, 2014)

Fungicide application can lead to toxic residues on the fruits (Wedge *et al.*, 2013). Depending on the time of application, fungicides may reduce pollination and cause malformed fruits due to the time of application (Ruby *et al*., 2017). The sources of grey mould are mycelia in dead strawberry leaves, mummified strawberry fruits, and straw mulch, and weed (Garrido *et al*., 2011). Biological methods can be developed as an alternative to chemical fungicides for fruit rot control (Jacometti *et al*., 2010). Leaves and flowers at the bud stage are highly susceptible to infection by gray mould; therefore, the beneficial microorganism should be used in the early time of the growing season.

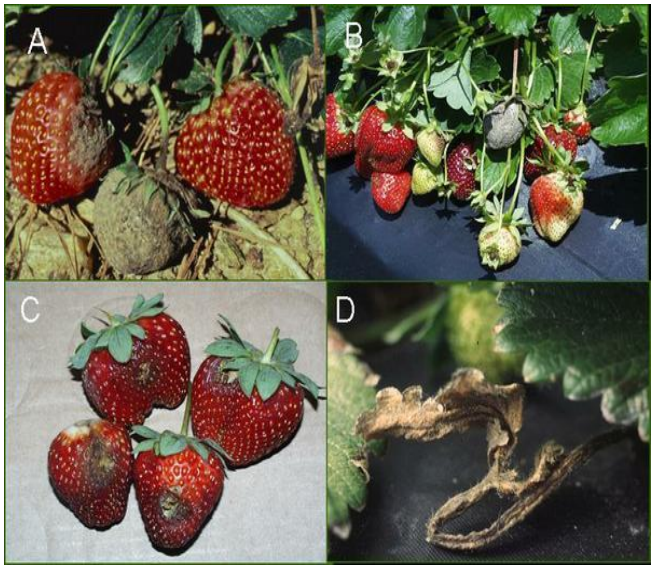

Figure 2. *Botrytis cinerea* symptoms in strawberry fruits

Source: Haidar *et al.* (2016)

2.6 Chemical Control of Gray Mold (*Botrytis cinerea*) in Strawberry

The control of this pathogen remains a challenge and is still based on multiple applications of fungicides. Chemical control is effective and efficient but, at the same time, can lead to the development of chemical residue in fruits, pathogen resistance, phytotoxicity to other organisms or environmental and public health problems (Cosseboom *et al*., 2019). To minimize these factors and to comply with food safety standards, there is an increased interest in bio-ecology studies of this pathogen and a worldwide trend to explore new alternatives to synthetic fungicides.

2.7 Biological Control of Grey Mould (*Botrytis cinerea*) in Strawberry

The difficulty in controlling *Botrytis cinerea* has necessitated the search to find alternative methods, which include biological control (Nicot *et al*., 2016). Biological control is an alternative to reduce botrytis infections and is successful in many other crops (Nicot *et al*., 2016). Currently, several bio control agents have been recognized and are available as bacterial agents, for example, *Pseudomas, Bacillus*, and *Agrobacterinum*, and as fungal agents such as *Aspergillus*, *Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida*, and *Coniothyrium* (Sylla *et al*., 2015). Strawberry flowers are important sites of primary *B. cinerea* infections. After the establishment of infections, the pathogen remains quiescent until harvest. Postharvest control of previously established *B. cinerea* infections through biological controls is considered limited. For this reason, it is crucial to prevent infections of *B. cinerea* during flowering (Droby & Lichter, 2014; Ippolito & Nigro, 2016). This was confirmed by Lima *et al*. (2013), who found the high activity of *A. pullulans* and *Candida oleophila* against *B. cinerea* during storage when biological control agents were applied during flowering, whereas only low biological control agents activity was observed when biological control agents were applied immediately before harvest.

Among these biocontrol agents *Trichoderma* spp. is one of the most versatile biocontrol agents which has long been used for managing plant pathogenic fungi. A previous study found that seed rot, damping-off and root rot of sunflower and mug bean both caused by *Sclerotium rolfsii* can be prevented while at the same time plant growth is enhanced when plants are treated with the conidial suspensions of *Trichoderma* spp. (Contreras *et al* ., 2016). In addition, the pathogenic fungal growth of *Ganoderma* is inhibited by *T. harzianum*and *T. virens* (Naher *et al*., 2012).

Trichoderma species have been identified as potential bio control agents of many plant pathogenic fungi (Herrera-Parra *et al*., 2017). *Trichoderma harzianum*is one of the most studied members within this genus as a biological control agent against an array of plant pathogenic fungi, including *B. cinerea* (Chen *et al*., 2016*).* Recent studies have shown that *T. asperellum* is effective and promising bio control agent in the control of several soil-borne fungi, such as *Phytophthora drechsleri* (Hassan *et al*., 2020), *Phytophthora capsici* (Kim & Knudsen 2013) and Fusarium *oxysporum (*Ommati & Zaker, 2012). The antagonism of *Trichoderma spp*. to *Fusarium solani* and other soil-borne has been reported (Chen *et al*., 2016). *Trichoderma harzianum* and *T. viride* have shown prospects in the control of soil-

borne pathogens. For many years, Trichoderma isolates have been used in different fields of production and protection in agriculture (Bendahamane *et al*., 2012). *Trichoderma spp*. has no known harmful effects on human or animal health or the environment (Schuster & Schmoll, 2010). Many companies use these fungi to produce biological plant protection products or plant growth promoters (Kowalska, 2011).

2.8 Use of *Trichoderma asperrellum* in Grey Mould (*Botrytis cinerea*) Control in Strawberry

Biological control involving Trichoderma spp. operates by way of mycoparasitism, antibiosis, and competition. Antagonistic abilities of Trichoderma spp. are a combination of several mechanisms including direct mycoparasitism, which involves the production of cell-wall degrading enzymes (Qualhato *et al*., 2013). Trichoderma spp. secrete cell wall degrading enzymes like chitinases and volatile and non- volatile compounds that enter the cell in the form of signal and triggers secondary messengers and altering the metabolic pathway of the pathogen (Komy *et al*., 2015). Most Trichoderma strains produce volatile (Mukherjee e*t al*., 2012) and non-volatile toxic secondary metabolites, among this production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid, and others have been described (Vinale *et al.*, 2012). They are frequently associated with bio control activity and the promotion of plant and root growth (Saba *et al*., 2012).

Trichoderma spp. is a fungus present in nearly all agricultural soils (Zhang *et al*., 2013). It has been demonstrated that *Trichoderma* strains can survive in the aerial part of the plants for long periods (Perello *et al*., 2013) and can act as antagonists of foliar fungal pathogens in a wide range of crops (Elad, 2016). Some authors have reported that biological control is a promising strategy for managing. Fusarium wilt pathogen of tomato (Perello *et al*., 2009). *Trichoderma* spp. are bio control agents effective against fungal phytopathogens. They can act indirectly, by competing for nutrients and space, modifying environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Vinale *et al*., 2012).

Trichoderma asperellum inoculum when used as a substrate in strawberry, the incidence, and severity of *Phytophtora cactorum* and *Verticilium dahliae* septoria is reduced (Martinez *et al.*, 2009). The same activity has been reported for alternalia disease in tomato when other beneficial leaf colonizing organisms like *Cryptococcus laurentii, Candida tenuis, C. oleophila* and *Pseudomonas putida* are used (Segarra *et al*., 2013). The treated plants may

produce natural substances, which induce defence reaction against pathogen infection and help the host plant to take up more nutrients from the soil. Production of natural substances, which induce plant defence, has been described for *Pythium oligandrum*. *Pythium oligandrum* induces defence reaction in a plant by phytohormones stimulation, which is involved in the resistance mechanisms against the pathogen (Komy *et al*., 2015).

2.9 Botanical Control of Grey Mould (*Botrytis cinerea*) in Strawberry

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal, and insecticidal properties under laboratories and field trials (Decorato *et al*., 2017). Plant metabolites and plant-based pesticides provide better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Tamuli, 2014). The antimicrobial properties of plant extracts from various species have proven to affect fungal development in vitro and in vivo (Bhutia *et al*., 2015). Spore formation and germination, mycelia growth, and infection can sometimes be stimulated or inhibited by plant extracts (Decorato *et al*., 2017).

Among alternative methods of grey mould control, the use of plant extracts is characterised by lack of toxicity for humans and environment, biodegradable activity selectivity, and a great variety of chemical composition, with a large variety of secondary metabolites (Choudhury *et al*., 2018). Plants extracts of the following crops, *S. hortensis*, *A. sativum*, *H. officinalis*); were found to be effective in controlling *Botrytis cinerea* in blackcurrant crop (Sesan *et al*., 2015). According to Senhaji *et al.* (2014) *A. imbricatus and P. mauritanica* extracts were able to control *Botrytis cinerea* in tomato.

2.9.1 Mexican Sunflower (*Tithonia diversifolia*)

Tithonia, commonly known as Mexican sunflower is an invasive, perennial weed, growing aggressively along road paths, abandoned farmlands, and hedges (Dahunsi *et al*., 2017). Tithonia is a shrub and belongs to the family Asteraceae. Tithonia originated from Mexico and it is now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa Tithonia is well established in Kenya (Jamal *et al*., 2000).

The reported uses of tithonia include as a fodder (Chagas *et al.*, 2012), animal feed (Obua *et al*., 2012), fuel wood compost, land demarcation, soil erosion control, building materials and shelter for poultry **(**Shackleton *et al*., 2019**)**. Besides addition, extracts from tithonia plant parts reportedly protect crops from termites contain chemicals that control insects diseases, and nematodes (Njenga *et al*., 2019). Extracts of Tithonia also have medicinal value for the treatment of hepatitis and control of amoebic dysentery (Tagne *et al*.,

2018**)**. Tithonia is being used as a source of nutrients for crops in countries such as Malawi, Zimbabwe, Kenya, and Nigeria (Adeniyan et *al*., 2008; Ajao *et al*., 2017). Liasu and Atayese (2006) found that soil under Tithonia had higher pH, porosity, moisture content, N, P, K, Ca, mycorrhizal fungi spores, earthworm casts, and lowers bulk density compared with bare soil.

Figure 3. *Tithonia diversifolia* plant

Source: Dahunsi *et al*. (2017)

2.9.2 Mineral Nutrient content of Tithonia

According to Hafifah *et al*. (2016) the quantity of nutrients found in tithonia is significantly higher than those found in synthetic fertilizers. *T. diversifolia* plays an important role in crops nutrition and control due to its biofertilising (Kandungu *et al*., 2013).The moisture content of tithonia leaves is estimated to be 84%. Before the plant flowers, Tithonia leaves on average contain the following nutrients: Nitrogen (N) 3.17%, Phosphorus (P) 0.3%, Potassium (K) 4.1%, Calcium (Ca) 2.0%, Magnesium (Mg) 0.3% (Hafifah *et al*., 2016). Olabode *et al*. (2007) also reported, chemical properties of *Tithonia diversifolia* as follows: organic matter, N, P, K, Ca, Mg, C, and C/N 24.04%, 1.76%, 0.82%, 3.92%, 3.07%, 0.005%, 14.00%, and 8:1, respectively.

2.9.3 Antifungal Properties of *Tithonia diversifolia*

Studies have shown the effectiveness of plant extracts in controlling plant diseases in the field. *Tithonia diversifolia* has been used widely because it has antifungal and anti-bacteria properties and has shown a broad spectrum due to the presence of antimicrobial compounds (Ogunfolakan, 2010). A study conducted by Kereru *et al*. (2010) showed that extracts made from *Tithonia diversifolia* were able to control *Fusarium oxysporum*. The inhibitory effects of *Tithonia diversifolia* was explained by Agboola *et al*. (2016) who

reported that Tithonia was able to inhibit mycelial growth of fungal pathogens such as *C. lunatus, F. lateritium,* and *F. solani.* The ability of Tithonia to control fungal pathogens in plants is due to prescene Thithoniaquinone A and tithoniamide B, psoralen, and Iquebrachitol which are fungicidal and antibactrial.

Extracts from several Asteraceae as well as *Tithonia. diversifolia,* were effective in inhibiting of the mycelia growth of *Trichophyton mentagrophytes* and *F. oxysporium* (Agboola *et al*., 2016). Several studies exhibited the efficiency of plant extracts in controlling plant diseases in the field. Jimoh *et al.* (2016) reported that Cercospora leaf spot disease of sesame (*Sesanum indicum L*.) was controlled with plant extracts including extract of *Tithonia diversifolia*. Field evaluation of leaf extracts of some Asteraceae especially *Tithonia diversifolia* were effective in the control of leaf spot disease of sweet potatoe. Jimoh *et al.* (2016) reported that *Cercospora* leaf spot disease of sesame (*Sesanum indicum* L.) was controlled by using plant extracts including *Tithonia diversifolia*. Field evaluation of leaf extracts of some Asteraceae especially *Tithonia diversifolia* were effective in the control of leaf spot disease of sweet potatoes in Abraka (Ilondu et *al*., 2014).

According to Ilondu *et al*. (2014), *Tithonia diversifolia* was found to have more than 10 compounds with antifungal properties and inhibited mycelial growth of fungal pathogens. *Tithonia diversifolia* leaves are used for the treatment of various diseases including skin diseases and possess many chemical components (Omokhua *et al*., 2018). These antifungal properties help in controlling *Botrytis cinerea* in strawberry. Several reports also indicate that *Tithonia diversifolia* controls fungal diseases in many plants as shown in the table below (Linthoingambi *et al*., 2013).

Table 3. Antifungal properties of *Tithonia diversifolia*

Fungal species	Source of isolation	Type of infection
Alternaria alternate	French bean leaf	Leaf spot disease
AlternariasolaniSorauer	Potato leave	Early blight disease
Aspergillus flavus	Groundnut seed	Aflatoxin contamination
Aspergillus niger	Groundnut seed	Black mould disease
Curvularia lunata	Rice grain	Grain discoloration
Drechslera oryzae	Rice grain	Brown spot disease
Fusarium oxysporum	Tomato root	Wilt disease
Penicillium expansum	Apple fruit	Soft rot
Penicillium italicum	Lemon fruit	Blue mould rot
Trichoderma viride	Field soil	Bio control agent

Source: Linthoingambe *et al*. (2013)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental Site

The study was carried out at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0°23'South, longitudes 35° 35'East in the Lower Highland III Agro-Ecological (LH3) at an altitude of approximately 2,238 meters above sea level. Average maximum and minimum temperature range from 19°C to 22°C and 5°C to 8°C respectively, with a mean annual rainfall of 1000 mm. The soils are predominately vitric mollicandosols (Jaetzold *et al*., 2006).

3.2 Materials and Lab Equipment

Disease-free strawberry (*Fragaria× ananassa var duch*) splits were obtained from a farmer in Thika. *Trichoderma asperrellum* was obtained from Real IPM Company (Thika) while *Tithonia diversifolia* was obtained from farms in Murang'a.

Equipment which were used during the experiment were electronic balances (model: Hangping JA 2003), incubators, sprayers, Petri dishes, refractometer (RHW Refractometer, Optoelectronic Technology Company Ltd. UK), measuring cylinders and farming tools were obtained from Horticulture Research and Teaching Field laboratory.

3.3 Preparation of Pathogen Inoculum

Botrytis cinerea was isolated from infected strawberry leaflets discs. The infected leaf discs were cut into small pieces ($1cm^3$) and surface sterilized using 70% ethanol for two minutes. The infected leaf discs were rinsed several times in distilled sterilized water and dried using sterile filter papers. After rinsing, the leaf discs were placed in Petri dishes containing potato dextrose agar (PDA) with 100 mg/L streptomycin for culturing at 24-30 °C for 10-14 days, under a 12:8 h (light: dark) photo regime. The developing mycelia were transferred to fresh PDA medium for two weeks. The pathogen was identified using spore morphology. Conidia suspension of the fungus was prepared by flooding the two-week sporulation culture with one litre sterilized distilled water containing one drop of Tween 20 (Fisher Scientific, Pittsburg, PA), (Janisiewiez, 1998**)**. Conidia suspensions were then filtered through a sterile cheesecloth. Dilutions were adjusted to 1×10^4 spores per mL through serial dilutions.

3.4 Artificial Inoculation

All experiments were conducted under artificial inoculation in the field where *Botrytis cinerea* conidia suspension (1×10^{4} conidia per mL) was sprayed on each plant leaf until runoff four weeks after planting. The open flowers were inoculated with 1×10^{4} conidia per mL. Plants were sprayed 3 times, at an interval of 3 days. The inoculation was done late in the evening to prevent moisture loss. Samples of the infected plant parts were cultured, and the pathogen was isolated to confirm the presence of *Botrytis cinerea.*

3.5 Preparation of Materials of *Tithonia diversifolia* Extract and *Trichoderma asperellum*

Tithonia diversifolia biomass was obtained by cutting the foliage above the ground, which was then be cut into small pieces. Twenty kilograms of the cut foliage was mixed with 200 liters of distilled water to maintain the required ratio of leaf tea 1:10 (Altierri *et al.,* 1986; KATC & SCC, 2007). The mixture was stored in 200-liter plastic container which was covered using sisal sack to reduce volatilization. The container was held then under room temperature throughout the storage time.

The mixture was agitated once a day for three weeks to provide a sufficient aerobic environment. After incubation, the mixture was sieved using a white cloth and the filtrate was ready for application. Tithonia was applied at four concentrations; 0mL, 250mL, 500mL and 750mL per liter of water. *Trichoderma asperrelum* was obtained from Real IPM Company and the (Trade name: Real Trichoderma). *Trichoderma asperellum* was applied using a 20 liters backpack sprayer at three concentrations; 0mL, 40mL, and 80mL per 20 litres of chlorinated clean tap water.

3.6 Experimental Design and Treatment Application

The study was carried out as a factorial experiment in a Randomized Complete Block Design (RCBD) with 3 replications. There were two factors *Tithonia diversifolia* and *Trichoderma asperellum. Tithonia diversifolia* was applied at four concentrations (0mL/L, 250mL/L, 500mL/L, and 750mL/L) while *T. asperellum* was applied at three levels (0mL, 40mL and 80mL per 20 litres of water)) the blocks were used to block against soil factors to reduce errors. The experiment covered an area of 33.3 m by 5.6 m with individual blocks measuring 33.3 m by 1.2 m separated by a 1m path. Individual experimental units within a

block measured 1.2 m by 2.10 m with an inter-plot spacing of 0.5 m (Fig. 1). The treatments were applied two weeks after planting, during flowering, and one week before harvest.

Table 4. Treatment Combinations of *Tithonia diversifolia* and *Trichoderma asperellum*

***T.asperellum* in millilitres**	***Tithonia diversifolia* extracts in mL/L**			
	Td1 (0mL)	Td2 (250mL)	Td3 (500mL)	Td4 (750mL)
Ta1(0mL)	Ta1Td1	Ta1Td2	Ta1Td3	Ta1Td4
Ta2(40mL)	Ta2Td1	Ta2Td2	Ta2Td3	Ta2Td4
Ta3(80mL)	Ta3Td1	Ta3Td2	Ta3Td3	Ta3Td4

3.7 Land Preparation and Crop Maintenance

The field was manually dug to approximately 20 cm depth and prepared to a fine tilth using a rake. Well decomposed cattle manure was applied at the rate of 5 ton per ha during planting. Healthy and disease free splits of strawberry variety Chandler were planted at a spacing of 30 cm × 40 cm giving 20 plants per plot. Cultural management practices such as watering, weeding, and insect pest control was carried out uniformly in all plots to avoid variations. Weeding was done manually, while insects were physically removed from the strawberry plants. The cultural practices were carried out after planting until harvest.

3.9 Data Collection

Data collection for all variables commenced 28 days after planting and continued at intervals of 14 days up to 100 days after planting (DAP). At each instance of data collection, the mean for all variables from each replicate was computed. The mean number of the variables was determined by computing the means of the replicate mean.

3.10 Growth Variables

3.10.1 Plant height

The average height of six plants was measured in centimetres using a standard meter rule. The measurements were done from the crown level to the apex of the primary leaves. This was carried out after every two weeks starting at 28 days after planting until the first harvest.

3.10.2 Number of leaves per plant

Data on the number of leaves were recorded from six plants chosen at random from two rows in the centre of each plot. The results were expressed as leaf number per plant. These results were collected out after every two weeks until the first harvest

3.10.3 Number of flowers per plant

Data on numbers of flowers per plant were recorded from six plants chosen at random from two rows in the centre of each treatment. The results were then expressed as the average number of flowers per plant.

3.10.4 Fruit length

The length of ten randomly selected fruits from each treatment was measured in centimetres from the calyx plug to the pointed end or apex of the fruit. The measurements were done using an ordinary 30-centimeter ruler.

3.10.5 Fruit diameter

The diameter (latitude) of ten randomly selected fruits from each treatment was measured in centimetres using a vernier caliper. The diameter was taken at the full ripe stage.

3.11 Fruit Yield variables

3.11.1 Fruit weight

To determine the berry weight, ten berries from each treatment in tagged plants were randomly selected and the average weight of berry was measured using an electronic balance (model: Hangping JA, 2003). The results were then expressed as mean fruit weight in grams.

3.11.2 Number of fruits per plant

The number of fruits i.e., primary, and secondary, tertiary were counted from six plants at the time of fruit maturity and were expressed as the number of fruits per plant.

3.11.3 Total fruit yield per plant

The total fruit production in each treatment was recorded from six plants per plot and yield per plant were calculated and expressed in grams.

3.12 Quality variables

3.12.1 Total soluble solids

Total soluble solids of the fresh fruits were recorded by using a digital refractometer (RHWRefractrometer, Optoelectronic Technology Company Ltd.UK) at room temperature and expressed in degrees, Brix. The refractometer was cleaned with distilled water after each observation (Majidi *et al.*, 2011)

3.12.2 Ascorbic acid

Ascorbic acid content was determined by using 2, 6- Dichlorophenol-indophenol (AOAC, 1990). Ten grams of the fruit sample was extracted in 30mL of 5% oxalic acid in a mortar and pestle, and then filtered Whatman No.1 filter paper. Standard indophenol solution was prepared by dissolving 0.05g of 2, 6-dichlorophenolindophenol in distilled water, diluted to 100 mL, and filtered. The ascorbic acid standard solution was prepared by dissolving 0.05 of pure ascorbic acid in a small volume of 5% oxalic acid solution and then diluted to 250 mL with the same oxalic acid solution. Ten millilitres of the ascorbic acid standard solution was titrated with the indophenol solution to a slightly pink endpoint. Ten millilitres of oxalic acid was titrated as blank. The amount of ascorbic acid corresponding to 1 mL of indophenol solution was then calculated. Ten mL of the filtered sample extract was pipetted into a 50 mL flask and made to the mark with the 5% oxalic acid solution. The standard Indophenol

solution was used to titrate 10mL of the filtrate. The vitamin C content was calculated as a mg/100g sample (Majidi *et al*., 2011)

Calculation; Mg ascorbic acid/g or mL sample C=V× (DF/WT)

Where C= mg ascorbic acid V=mL dye used for titration of diluted samples

DF= dilution factor, WT=sample weight in g

3.13 Disease variables

Twenty fruits from each treatment were surface sterilized by dipping into 2% sodium hypochlorite for 2 min. Sterilized fruits were then put in plastic trays and stored at 10°C and 95% relative humidity for 10 days.

3.13.1 Disease Incidence

The percentage of decay incidence was identified using Koch's postulate. It was obtained by (i) considering the number of fruits that showed signs of decay over the initial number of fruits. The cumulative decay during storage was expressed as a percentage of infected fruits. The data was taken 10 days after storage.

$$Disease\ incidence(DI) in\ Fruits = \frac{Number\ of\ infected\ fruits}{The\ initial\ number\ of\ fruits} \times 100$$

3.13.2 Disease Severity

(ii) Disease severity on strawberry fruits surface

Disease severity on the berries surface were assessed basing on a scale of 0-5 where 0 = no infection, 1 = 1-5% berry surface affected, 2 = 6-15% berry surface affected, 3 = 16-50% berry surface affected and 4 = 51-95% berry surface affected 5- 100% berry surface affected. This scale is similar to those used by other researchers investigating the same disease organism, *Botrytis. cinerea,* on strawberries (Elad & Shtienberg, 1994; Archbold *et al.,* 1997). The percentage of fruit rot severity caused by *Botrytis cinerea* was estimated by the equation adopted by Hanounik (1988)

$$Disease\ Severity(DS)\% = \frac{\sum(NPC \times CR)}{NIP \times MSC} \times 100$$

Where:

CR= Class rate (scale based on *Botrytis cinerea* symptoms)

NPC=No of strawberry fruits infected by botrytis in each class rate

NIP=No of fruits in each plot

MSC=Maximum severity class rate of infected strawberries fruits

Data Analysis

Data was analysed using GenStat 15th edition. The proc-univariate procedure was used to check for normality of the data before analysis, and arcsine data transformation was done for disease incidence and severity... All numerical data were subjected to analysis of variance (ANOVA) at $P \leq 0.05$ and means for significant treatments were separated using Tukey's honestly significant difference test at $P \leq 0.05$.

3.8 Experimental Model

In the study growth, yield, quality and disease incidence and severity parameters were considered dependent variables while *Tithonia diversifolia* extracts and *Trichoderma asperellum* were considered independent variables. The model for the experiment was as follows:

$$Y_{ijk} = \mu + T_i + A_j + \beta_k + (TA)_{ij} + \varepsilon_{ijk},$$

$i = 1, 2, 3, 4; j = 1, 2, 3; k = 1, 2, 3$

Where,

Y_{ijk} - An individual observation from an experimental unit receiving the ith level of *Tithonia diversifolia*, jth level of *Trichoderma asperellum* in the kth block.

μ - The overall mean (An unknown constant).

T_i - The effect of the ith level of *Tithonia diversifolia*

A_j -The effect of the jth level of *Trichoderma asperellum*

β_k - The effect of the kth block.

$(TA)_{ij}$ - The interaction effect between the i^{th} level of *Tithonia diversifolia*andjth level of *Trichoderma asperellum*

ε_{ijk} - Random error component associated with response from an experimental unit receiving the i^{th} level of *Tithonia diversifolia*andj^{th} level of *Trichoderma asperellum* and in the k^{th} block. Is assumed to be independent and normally distributed with zero mean and a common variance σ.

CHAPTER FOUR
RESULTS

Results obtained in this study are presented in this chapter following the order: growth variables, fruit yield variables, disease variables, and quality variables.

4.1 Objective 1: Effect of *Tithonia diversifolia* and *Trichoderma asperellum* concentration on growth and yield of strawberry

4.1.1 Plant height

The application of *Tithonia diversifolia* and *Trichoderma asperellum* significantly influenced plant height throughout the growing period. However, application of *Trichoderma asperellum* and *Tithonia diversifolia* did not have a significant ($P \leq 0.05$) effect on the plant height at 28DAP of growth (Table 5). *Trichoderma asperellum* 80mL and *Tithonia diversifolia* at 750mL produced the tallest plants compared to untreated plants. There was a significant ($P \leq 0.05$) interaction effect of *Tithonia diversifolia* and *Trichoderma asperellum* from week six of growth in the experiment.. The untreated plot produced the shortest plants compared to treated plants at 42DAP. There was a significant ($P \leq 0.05$) difference in plant height at 56DAP 70DAP and 84DAP in both trials (Table 5). Treated plots produced the tallest plants compared to untreated plots in all sampling dates. *Trichoderma asperellum* 40mL and *Tithonia diversifolia* 500mL had taller plants compared to other rates. At 84DAP plant, height was significantly($P \leq 0.05$) affected by the treatments with *Trichoderma asperellum* 80mL and *Tithonia diversifolia* 750mL producing the tallest plants (26.48cm) while control produced the shortest plants (17.63) (Table 5).

Table 5. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on Plant height (cm)

***Trichoderma asperellum* Concentration (mL)**		***Tithonia diversifolia* concentration (mL)**			
		0	250	500	750
		Height in cm			
	0	5.05c	5.63abc	6.03abc	7.367a
28DAP	40	6.30abc	5.86abc	6.20abc	6.47abc
	80	5.45bc	5.62abc	6.83ab	6.53b
42DAP	0	8.08d	8.92c	10.77b	11.05b
	40	9.38c	9.57c	11.48ab	11.40ab
	80	9.18c	9.02c	10.70b	11.87a
56DAP	0	11.85f	12.77e	13.70cd	14.55ab
	40	12.85e	13.08de	13.87bc	14.53ab
	80	13.52cde	13.05de	13.85bc	14.87a
70DAP	0	15.28d	15.27d	15.75cd	16.10bcd
	40	15.68cd	16.07bcd	16.58bc	16.57bc
	80	16.25bcd	16.08bcd	17.03b	18.53a
84DAP	0	17.63e	18.48de	20.15c	22.40b
	40	18.47de	18.78cde	19.98cd	24.97a
	80	18.48de	18.90cde	21.82b	26.48a

*Means followed by the same letter within a trial sampling date are not significantly different according to Turkey's HSD test at $p \leq 0.05$.DAP refers to days after planting

4.1.2 Number of leaves

Application *Trichoderma asperellum* and *Tithonia diversifolia* significantly (P≤0.05) influenced the number of leaves per plant throughout the growth period. The highest number of leaves were recorded in treated plants (58.82) compared to control plants (43.63) (Table 6). Significant differences in the number of leaves were observed from 42 DAP in both trials. The number of leaves remained significantly (P ≤0.05) higher at 56DAP, 70 DAP and 80DAP in treated plants compared to control plants in both trials. *Trichoderm asperellum* at 80mL and *Tithonia diversifolia* at 750mL produced plants with the highest number of leaves (58.82) 84 DAP.

Table 6 .Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on number of leaves

Trichoderma asperellum concentration (mL)		*Tithonia diversifoliar concentration (mL)*			
		0	250	500	750
		Number of leaves per plant			
28DAP	0	10.97f	11.52e	12.52c	12.73b
	40	11.63def	11.85cde	12.62bc	12.95ab
	80	12.15bcd	11.83cde	12.33d	13.17a
42DAP	0	15.52d	16.07d	19.20b	20.10a
	40	15.62d	16.33d	19.05b	20.08a
	80	17.63c	17.98c	19.90ab	20.10a
56DAP	0	21.22e	22.65cd	27.57e	27.48ab
	40	21.87de	22.55d	27.23b	27.88a
	80	23.43c	22.65cd	27.53a	28.27a
70DAP	0	32.42d	33.55cd	37.03ab	38.70a
	40	33.33d	34.63c	36.67b	38.47a
	80	37.28a	34.83bc	37.20a	37.78a
84DAP	0	43.63g	43.98g	44.40fg	46.60e
	40	44.47f	44.75ef	46.60e	48.60d
	80	48.93d	51.33c	54.13b	58.82a

*Means followed by the same letter within a trial sampling date are not significantly different according to Tukey's HSD test at P≤0.05. DAP refers to days after planting

4.1.3 Fruit length

Interaction of *Tithonia diversifolia* and *Trichoderma asperellum* rates significantly (P≤0.05) influenced fruit length (Table 7). The longest fruits were recorded in treated plots compared to untreated plots. *Trichoderma asperellum* 40mL and Tithonia *diversifolia* 750mL produced fruits with a length of (6.03cm).There were significant differences in fruit length with *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL recording the highest fruit length (6.43). The lowest fruit length (2.36cm) was obtained in control

Table 7. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on fruit length in cm

Trichoderma asperellum concentrations (mL)	*Tithonia diversifolia* concentrations (mL)			
	0	250	500	750
	Fruit length in cm			
0	2.13e	3.03d	5.63c	6.36ab
40	2.73d	2.80d	6.16b	6.03bc
80	3.13d	3.20d	5.93bc	6.43a

*Means followed by the same letter within a trial sampling date are not significantly different according to Tukey's HSD test at p≤0.05.

4.1.4 Number of flowers per plant

Application of both *Tithonia diversifolia* and *Trichoderma asperellum* resulted in a significant ($p \leq 0.05$) increase in the number of flowers. The highest number of flowers were recorded at 750mL *Tithonia diversifolia* and 80mL *Trichoderma asperellum* (22.83) respectively) (Table 8). However, there were no significant differences in plots treated with both *Tithonia diversifolia* (750mL), *Trichoderma asperellum* (80mL) and *Tithonia diversifolia* (500mL) and *Trichoderma asperellum* (40mL) in the experiment. The untreated trials produced the lowest number of flowers. Plots treated with *Tithonia diversifolia* at 250mL and *Trichoderma asperellum* at 40mL were not significantly different from plots treated with *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 250mL (Table 8)

Table 8. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on the number of flowers per plant

Trichoderma asperellum concentration(mL)	*Tithonia diversifolia* concentration (mL)			
	0	250	500	750
	Number of flowers per plant			
0	8.32g	9.30f	19.92c	22.30a
40	10.60de	10.37e	20.70b	22.75a
80	11.03de	11.12d	22.55a	22.83a

*Means followed by the same letter within a trial sampling date are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.5 Number of Fruits per plant

The number of fruits per plant was significantly (P≤0.05) affected by *Trichoderma asperellum* and *Tithonia diversifolia* application rates. The highest numbers of fruits were obtained in treated plots (12.80) while untreated plots registered the lowest number of fruits per plant (6.5) in the experiment (Table 9). The application of *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* 750mL produced plants with the highest number of fruits (12.80) at (p≤0.05) in the experiment. However, there was no significant differences between *Trichoderma asperellum* 40mL and *Tithonia diversifolia* 500mL and *Trichoderma asperellum* 80mL and *Tithonia diversifolia* 500mL in the trials. (Table 9).

Table 9. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on the number of fruits per plant

Trichoderma asperellum conc entration (mL)	*Tithonia diversifolia* concentration (mL)			
	0	250	500	750
	Number of fruits per plant			
0	6.55e	8.27d	12.62a	12.43b
40	9.78c	9.20c	12.30a	12.43a
80	10.70b	9.13c	12.20a	12.80a

*Means followed by the same letter within a trial sampling date are not significantly different according to Tukey's HSD test at p≤0.05.

4.1.6 Fruit diameter

Growing strawberry using *Tithonia diversifolia* and *Trichoderma asperellum* significantly (P≤0.05) influenced fruit diameter in the study (Table 10).The highest diameter (4.37cm) was recorded in treated plants compared to control plants (1.6cm) Application of *Tithonia diversifolia* at 750mL and *Trichoderma asperrellum* at 80 mL resulted in fruits with the highest diameter in the experiments (4.37cm) (Table 10). The untreated plots produced fruits with the lowest diameter in the experiments.. In the experiment *Trichoderma asperrellum* at 80mL and *Tithonia diversifolia* at 500mL (4.10cm) had no significant differences (P≤0.05) with *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL (4.37cm) (Table 10).

Table 10. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on fruit diameter in cm

Trichoderma asperellum concentration(mL)	*Tithonia diversifolia concentration (mL)*			
	0	250	500	750
	Fruit diameter in cm			
0	1.6e	2.47c	3.97a	4.05a
40	2.13d	2.47cd	4.12a	3.98a
80	2.88bc	3.05b	4.10a	4.37a

*Means followed by the same letter within a trial sampling date are not significantly different according to Tukey's HSD test at p≤0.05.

4.1.7 Fruit weight

Fruit weight was influenced by the application of *Trichoderma asperellum* and *Tithonia diversifolia* in the experiment study. Treated plants had fruits with significantly (P≤0.05) higher weight (16.10grams) compared to untreated plants (8.18grams) (Table 10). The weight of the fruits was significantly (P≤0.05) higher in plots treated with *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL. *Tithonia diversifolia* at 250mL and *Trichoderma asperellum* at 0mL produced fruits with the lowest weight (8.50grams) compared to other treatments. Untreated plots had the lowest weight in the experiments (Table 11).

Table 11. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on fruit weight in grams

Trichoderma asperellum concentration(mL)	*Tithonia diversifolia* concentration (mL)			
	0	250	500	750
Fruit weight in grams				
0	8.18f	8.50f	14.65d	15.28bc
40	9.80e	8.72f	14.73cd	14.78cd
80	9.65e	9.50e	15.58ab	16.10a

*Means followed by the same letters within atrial sampling date are not significantly different according to Tukey‟s Honestly Significant Difference Test at P≤0.05

4.1.8 Fruit yield per plant

Application of *Tithonia diversifolia* and *Trichoderma asperellum* influenced yield per plant in the experiment study. Significant (P≤0.0.5) higher fruit yields were obtained in treated plants compared to control (Table12). During the experiment, the highest yield per plant was recorded at *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL (199.5grams) (Table 12). Among other treatments *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 500mL resulted to highest yields per plant. The lowest yields were recorded in control plants in both experiments (53.9grams).In trial two, the means for *Tithonia diversifolia* at 750mL and *Trichoderma asperellum* at8 0mL were not significantly different from *Tithonia diversifolia* at 500mL and *Trichoderma asperellum* at 40mL.

Table 12. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on yield per plant in grams

Trichoderma asperellum concentration(mL)	*Tithonia diversifolia* concentration (mL)			
	0	250	500	750
	Yield per plant in grams			
0	53.9f	84.1de	188.9ab	187.3ab
40	96.9cd	80.1e	181.5b	186.6ab
80	103.8c	87.6d	193.6ab	199.5a

*Means followed by the same letters within a trial are not significantly different according to Tukey's Honestly Significant Difference Test at P≤0.05

4.2 Effect of *Tithonia diversifolia* and *Trichoderma asperellum* on Fruit Quality

The results on the effect of *Trichoderma asperellum* and *Tithonia diversifolia* on total soluble solids (TSSs) and ascorbic acid.

4.2.1Total soluble solids

From the results, the application of *Tithonia diversifolia* and *Trichoderma asperellum* influenced the amount of Total soluble solids in the experiments (Figure 4). Untreated plants produced fruits with the lowest amount of Total soluble solids (7.97 brix %) compared to treated plants (13.28 brix %) in the experiments (Figure 4). The highest amount of total soluble solids was recorded at *Tithonia diversifolia* 750mL and *Trichoderma asperellum* at 80mL in both trials though there were no significant differences (P≤0.05) between the means in all the experimental units. In the experiments control produced fruits with the lowest Total soluble solids.

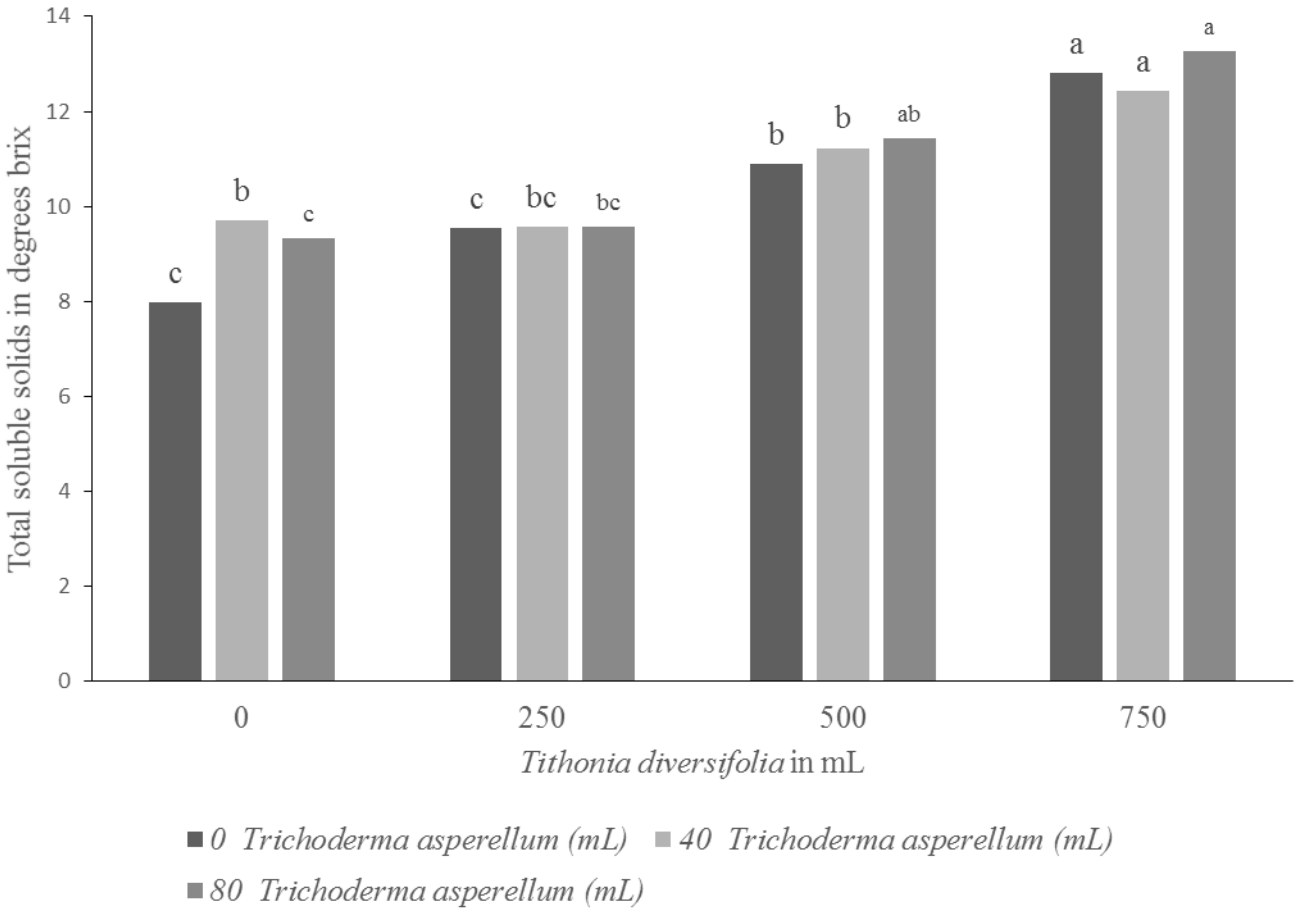

Figure 4. Effect of *Tithonia diversifolia* and *Trichoderma asperellum* on Total soluble solids in brix %. Means followed by the same letter are not significantly different according to Tukey's HSD test at p≤0.05.

4.2.2 Ascorbic acid

The ascorbic acid content in strawberry fruit was significantly influenced by the application of *Tithonia diversifolia* and *Trichoderma asperellum* rates (Figure 5). Ascorbic content was significantly (P≤0.05) higher in treated fruits compared to fruits from control plots. The highest level of ascorbic acid was recorded at *Tithonia diversifolia* at 750mL and *Trichoderma asperellum* 80mL (55.12 mg/100g). *Tithonia diversifolia* at 0mL and *Trichoderma asperellum* at 0mL produced fruits with the lowest ascorbic content (38.75mg/100g).(Figure5).. There was a significant (P≤0.05) difference between the means in the experiments.

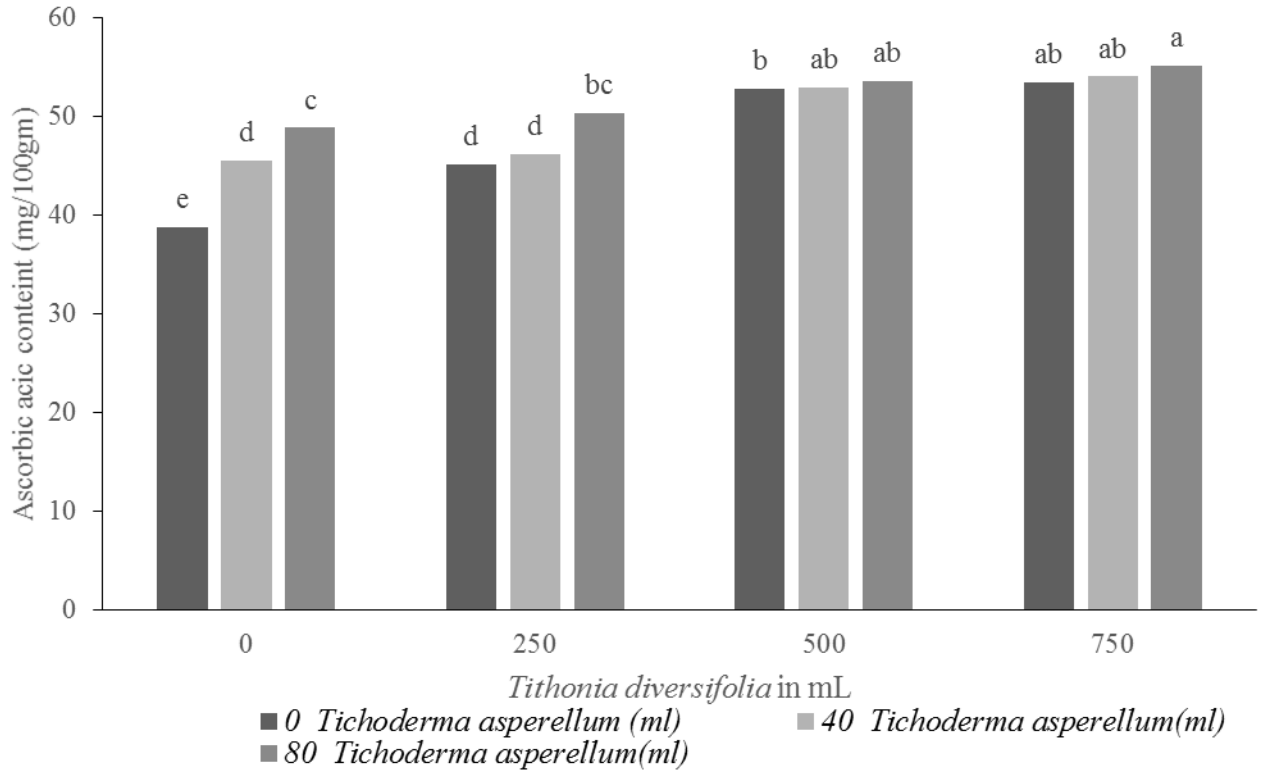

Figure 5. *Effect of Tithonia diversifolia and Trichoderma asperellum* on ascorbic acid content in (mg/100g).Means followed by the same letter are not significantly different according to Tukey's HSD test at p≤0.05 .

4.3 Effects of *Trichoderma asperellum* and *Tithonia diversifolia* Concentrations on Disease severity and Incidence

The results on the effect of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on *Botrytis cinerea* severity and incidence are outlined in Figure 6 below.

4.3.1 Disease Severity

Disease severity was significantly (P≤0.05) affected by the application of *Tithonia diversifolia* and *Trichoderma asperellum* rates. From the results, *Botrytis cinerea* severity varied depending on the amount of *Trichoderma asperellum* and *Tithonia diversifolia* applied (Figure 6). High rates of *Trichoderma asperellum* was observed to be effective for management of *Botrytis cinerea* in the experiments but it depended on the levels of *Tithonia diversifolia* applied. The disease severity index was significantly (P≤0.05) high in untreated fruits (89.04%) as compared to treated fruits (12.07%) in the experiment. Disease severity indexes was lowest at *Tithoinia diversifolia* 750mL and *Trichoderma asperellum* at 80mL in both trials(Figure 6).

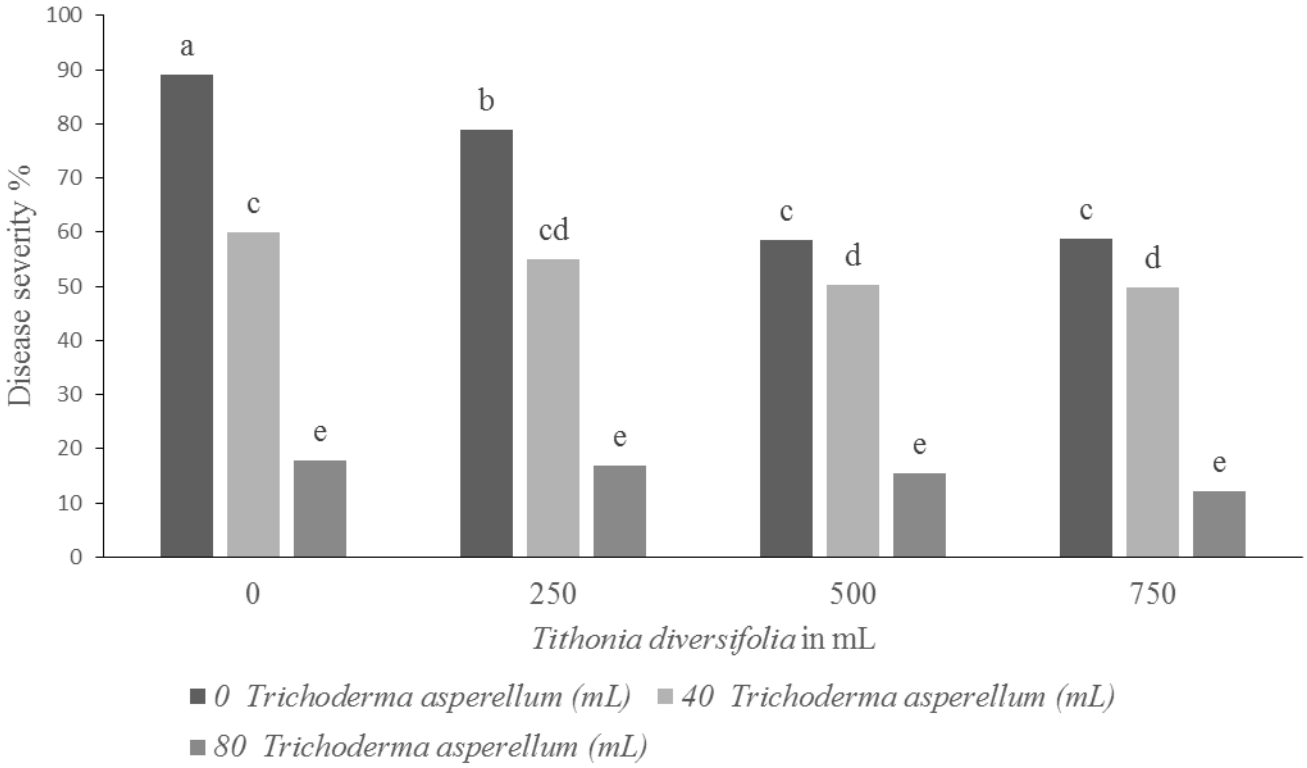

Figure 6. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on disease severity percentage .Means followed by the same letter are not significantly different according to Tukey's HSD test at p≤0.05

4.3.2 Disease Incidence

Trichoderma asperellum and *Tithonia diversifolia* had significant ($P \leq 0.05$) effects on disease incidence percentage in strawberry fruits (Figure 7). *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL had the lowest disease incidence (16.49%). Untreated fruits had the highest disease incidence (85.72%). High rates of *Trichoderma asperellum* was observed to be effective for the management of *Botrytis cinerea* but it depended on the levels of *Tithonia diversifolia* applied (Figure 7).

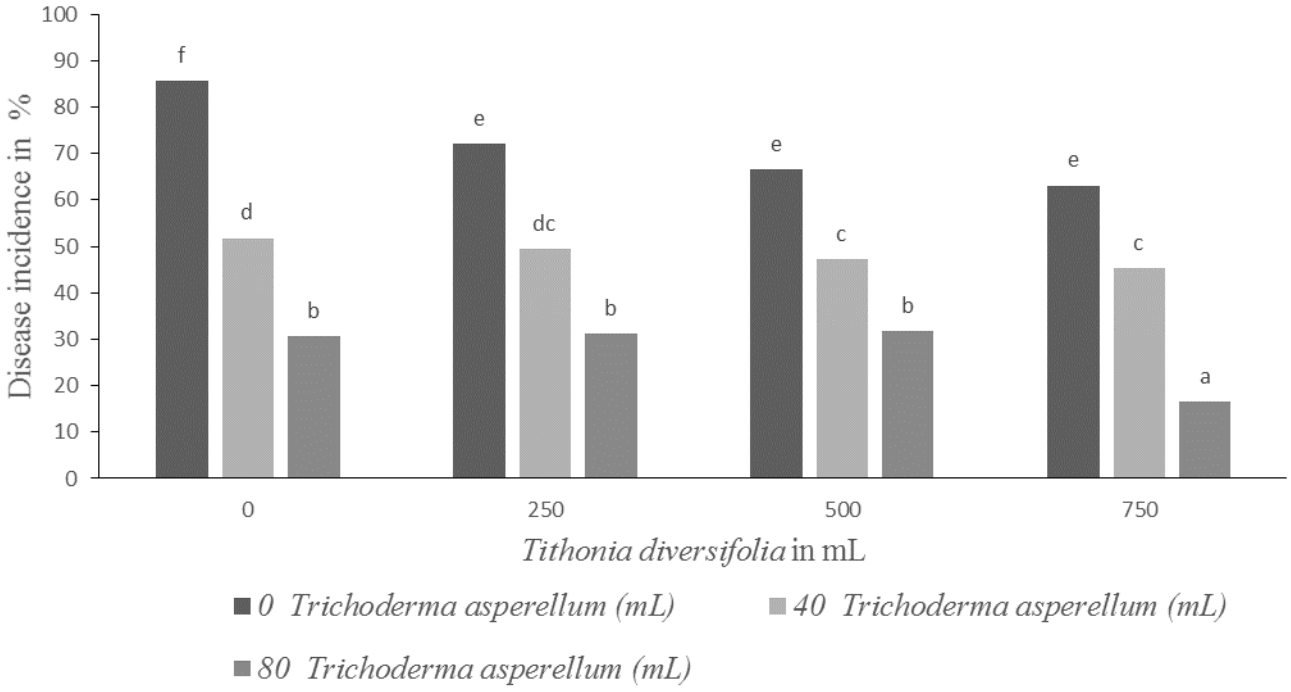

Figure 7. Effects of *Trichoderma asperellum* and *Tithonia diversifolia* concentrations on disease incidence percentage. Means followed by the same letter within a trial are not significantly different according to Tukey's HSD test at $p \leq 0.05$.

.

CHAPTER FIVE

DISCUSSIONS

This chapter presents a discussion of the results. The layout of this chapter follows sequentially the order in which the results were presented in chapter four of this document.

5.1 Effects of different concentrations of *Tithonia diversifolia* extracts and *Trichoderma asperellum* on growth and fruit yield of strawberry

5.1.1 Plant Height

In the present study, a combination of *Trichoderma asperrellum* and *Tithonia diversifolia* significantly enhanced plant growth giving taller strawberry plants compared to control. According to Elshahawy *et al*. (2019) and Zaghloul *et al*. (2017), *Trichoderma asperrellum* produces phytohormones, vitamins and solubilizing minerals, which inhibit pathogen growth, which leads to stronger and healthier plants consequently favouring an increase in plant height. *Trichoderma asperellum* was found to contribute to plant height due to the production of auxins, which stimulates plant growth (Contreras-Cornejo *et al*., 2009). These arguments support the findings of the current study. The response of strawberry plants to *Trichoderma asperellum* observed in this study is also supported by that of Mastouri (2010) who reported the highest growth measured in terms of plant height in tomato cultivated using *Trichoderma asperellum* compared to control. Similar results were observed by Fontenelle *et al*. (2011) who found positive results on the growth and development of seedlings in vegetable and non-vegetable crops grown with trichoderma species. *Trichoderma asperellum* contributed to an increase in height in tomatoes grown under different biotic and antibiotic stress (Mastouri, 2010). Hussain *et al*. (2011) findings reported that trichoderma had significantly additional and promoting effects on vegetative and qualitative traits of cut flowers, bulbs, and tulip. The synergistic effect between the microbial rhizosphere and bio control agent increases the activity of *Trichoderma asperellum* which leads to plant growth (Hajieghrari *et al*., 2016).

Several studies have documented the plant growth stimulation effect of trichoderma species and other microbes in crops such as bean (Scala *et al*., 2007), tomatoes (Ozbay*et al*., 2004), pea (Bisen *et al*., 2016). In the present study, *Tithonia diversifolia* produced the tallest plant compared to the control. The enhancement of height in this study was as a result of high levels of major nutrients (NPK) in tithonia extracts (Jeptoo *et al*., 2013). Similar results were

observed by Mustonen (2012) who reported significant responses in rice, maize, and vegetable crops to *Tithonia diversifolia* application. According to Babajide *et al.* (2012) *Tithonia diversifolia* was able to increase the plant height in sesame. Similar results were observed by Guong *et al.* (2010) who reported that tithonia amendment led to an increase in soil organic matter content, available nitrogen and phosphorous, cation exchange capacity, percentage base saturation, soil respiration, soil aggregate stability as well as reduce soil compaction. Green leaf biomass of tithonia contains 3.5% N, 0.37% P, and 4.1% K on a dry matter basis *(*Jamal *et al.,* 2000). Similar to this finding, Babajide *et al.* (2012) reported that tomato plant height increased with increasing compost rate.

5.1.2 Number of Leaves

In the present study, the use of *Trichoderma asperellum* and *Tithonia diversifolia* significantly affected the number of leaves in both trials. A combination of *Trichoderma asperellum* and *Tithonia diversifolia* increased the number of leaves compared to control plants. The findings of this study are supported by those of (Bae *et al*., 2011; Biljana *et al*., 2012; Ramot *et al.,* 2004) who also observed that *Trichoderma asperellum* increased plants growth and vigour in biotic and abiotic stress. Trichoderma not only attack other pathogenic fungi but also promote the growth of host plants (Chen *et al*., 2015; Hermosa *et al*., 2012). Its mode of actions includes antibiosis, mycoparasitism, and competition for nutrients and space (Bisen *et al*., 2015; Keswani *et al*., 2014). This particular group of fungi induces stress tolerance in host plants through the improved root and shoot development, inorganic nutrients solubilization, and sequestration, pathogenic enzymes inactivation and inducing systemic resistant (Bisen *et al*., 2016; Scala *et al*., 2007; Singh, 2014; Pill *et al*., 2009).

In the present study, strawberry plants treated with trichoderma exhibited more growth in terms of an increase in the number of leaves. These results were similar to those of Mastouri *et al*. (2010) who obtained a higher number of leaves in tomato after treating with *Trichoderma asperellum. Trichoderma asperellum* can induce auxin production in plants, which leads to an increase in plant growth, which results to the higher number of leaves (Conteras *et al*., 2009). The leaf number in strawberry depended on application rates of *Tithonia diversifolia* rates. According to Dauda *et al*. (2008), tithonia is rich in macronutrients such as N, P, and K, which leads to vigorous growth in plants. These results are similar to those of Aguyo *et al*. (2001) which showed a significant effect on watermelon growth to different rates of tithonia. Liasu *et al*. (2008) observed similar results in okra where

tithonia increased the number of leaves. A combination of tithonia and trichoderma led to the high number of leaves due to production of nutrients and growth hormones respectively.

5.1.3 Number of Flowers

The number of flowers per plant were significantly affected by the application of *Trichoderma. asperellum* and *Tithonia. diversifolia* rates. More flowers were observed in treated plants compared to control. The efficiency of *Trichoderma* spp. as bio fertilizers have gained support from numerous studies showing that when applied to plant surfaces soil and seeds, increases the solubility of nutrients as well as the nutrient uptake capacity of the root. According to Lopez- Bucio *et al*. (2015). Trichoderma spp were found to produce compounds such as phytohormones which stimulate root growth, thus increasing the absorptive surface of plant roots. These phytohormones include gibberellins cytokinins and indole-3-acetic acid, which promotes flowering (Tjamos *et al*., 2010). This supports the findings of this study where *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750 mL had the highest numbers of flowers per plant. Kerroum *et al*. (2015) observed similar results where the application of *Trichoderma asperellum* increased the number of flowers in tomato plants.

Similar observations were reported by Tondje *et al.* (2007) and Deberdt *et al.* (2008) who observed a high number of flowers in the cocoa plants. According to Jayasundara *et al.* (2016) tithonia produces nutrients, which lead to vigorous growth in plants leading to the high number of flowers. This is in agreement with Ademiluyi (2012) who found tithonia to have a significant effect on the number of shoot yield in Okra, which resulted to the higher number of flowers. A combination of all these factors contributed to a higher number of flowers in strawberry in both trials as a result of tithonia and trichoderma.

5.1.4 Fruit Weight and Fruit Yield

In the current study treated plants yielded higher compared to untreated plants in both trials. *Trichoderma asperellum* at 80 mL and *Tithonia diversifolia* at 750 mL produced the highest yields compared to other treatments. According to previous studies, Trichoderma spp were found to be plant symbionts that confer positive effects on crop yield, plant growth, and stimulation of plant defense through the production of secondary metabolites (Hermosa *et al.*, 2012). Mazhabi *et al*. (2011) revealed that, trichoderma had additional and promoting effects on vegetative and qualitative traits of tulip bulbs and cut flowers.

Trichoderma spp influenced strawberry growth resulting to increase in the number of fruits (Meunchang *et al*., 2006; Molla *et al*., 2005). Similar results were reported by Hague *et*

al. (2012) and Uddin *et al*. (2017) who documented that application of Trichoderma significantly influenced the weight of fruits, the number of fruits and the total yield of tomato fruits. The results of this study supported the argument by Bal and Altintas (2006) that reported that various species of trichoderma had a positive effect on the growth and yield of various crops. *Trichoderma asperellum* increased yield in vegetable crops (Lei *et al*., 2015). Tondje *et al.* (2007) reported that *Trichoderma asperellum* was able to increase the number of the pod in the cacao plants.

Trichoderma species showed variable responses in cucumber, strawberry and soybean (Gonçalves *et al.*, 2018). Besides, it was reported that the application of Trichoderma spp dramatically increased the number of fruits per plant in pepper and tomato grown in the greenhouse than untreated control (Vinale *et al*., 2012). The current study supports these findings with strawberry yield being enhanced using *Trichoderma asperellum*. According to Sallam *et al.* (2007), trichoderma increased the green yield of bean compared to untreated plants. Elshahawy *et al.* (2019) and Zaghloul *et al*. (2017) reported that application of selected trichoderma spp significantly increased the number of fruits/plant, the weight of fruits and the total yield of tomato fruits.

The ability of *Tithonia diversifolia* extracts to increase fruit yield in this study was due to its nitrogen, potassium, and phosphorous content (Hafifah *et al*., 2016). The results of the present study were similar to those of Fahrurrozi *et al.* (2017) who observed an increase in carrot root yield with an increase in tithonia level. Tithonia can substitute for urea as N sources according to Opala *et al*. (2015*)* and as a complement of inorganic fertilizers in Kales (Mwangi & Mathenge, 2014). Aghofack-Nguemezi and Dzukam (2016) reported that extracts and powders of tithonia improved tomato growth and yield .Foliar application of tithonia increased pechay plant weight and height (Pena *et al*., 2013). These findings are in agreement with those of Aguyoh *et al*. (2010) who reported a significant and positively correlated increase in a total yield of watermelon with increasing application rates of *Tithonia diversifolia* with yields enhanced by between 8.5% and 31% in plants subjected to the highest level *Tithonia diversifolia* compared to the control.

1.5 Fruits Number

Fruit number significantly increased in treated plants compared to control. A higher number of fruits were observed after application of *Trichoderma asperellum* 80 mL and *Tithonia diversifolia* 500 mL in both trials. These results were similar to those of Zaghloul *et al*. (2017) who observed a higher number of fruits in tomato as a result of *Trichoderma asperellum*.This was in agreement with Vinale *et al.* (2012) who reported that *Trichoderma asperellum* resulted to increase in the number of fruits in pepper and tomato. The enhancement of fruits number was due to ability of trichoderma to convert unavailable nutrients into available form leading to higher yields (Meunchang *et al*., 2006) In respect, to this findings trichoderma species were reported to encourage growth resulting to higher yield in cucumber, loofer and cucumber plants (Huang *et al*., 2020).

In the present study, *Tithonia diversifolia* significantly affected the number of fruits in both experiments. Consistent with the findings of the present study, Setyowati *et al*. (2018) reported a higher number of curds in cauliflower after treatment with Tithonia. Similar to this finding, Babajide *et al.* (2008) reported that tomato plant yield increased with increasing in tithonia rate. Green leaf biomass of tithonia contains 3.5% N, 0.37% P and 4.1% K on a dry matter basis, which contributes to increasing in the number of fruits, and plant yields (Guong *et al*., 2010). An increase in the number of fruits in this study also resulted due to the availability of phosphorous in tithonia, which is a key element in fruiting (Muktamar *et al*., 2017). According to Fahrurrozi *et al.* (2017), P-content of tithonia was 0.87% which encourages root expansion to get nutrients from the soil as well as responsible for crop maturity According to Jamal *et al.* (2000), Tithonia is very rich in nutrients which results to plant growth and hence increase in fruit number per plant.

5.1.6 Fruit length and fruit diameter

Fruits were longest in plots treated compared to untreated plots in trials 1 and 2.Significant differences were observed in the combination with tithonia at 750 mL and trichoderma at 80 mL producing the longest fruits. The results were similar to those of Setyowati (2018) who observed an increase in cucumber length and diameter. This was as a result of high nutrient content in tithonia green biomass (Babajide, 2012). Jayasundara *et al.* (2016) observed an increase in the length of okra after the application of *Tithonia diversifolia*. The ability of *Trichoderma sperellum* to stimulate plant growth and yield resulted in longer and large fruits in strawberry fruits (Stringlis *et al.*, 2018). According to

Jayaraman *et al.* 2014 trichoderma spp can induce plant defence mechanism systems resulting in healthy and strong plants, which can produce health large fruits.

5.2 Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on Quality of strawberry fruit

5.2.1Total Soluble Solids and Ascorbic Acid Content

Application of *Trichoderma asperellum* and *Tithonia diversifolia* significantly influenced the amount of total soluble solids and ascorbic acid content in strawberry fruits. The enhanced total soluble solids and ascorbic acid content were observed following the application of higher levels of tithonia in this study could be attributed to the better levels of macronutrients and micronutrients required for growth and quality (Rashidi & Keshavarzpour, 2007). Strawberry total soluble solids and ascorbic acid content were enhanced by Potassium, which is the key element in influencing the quality of fruits and vegetables. Aguyo *et al.* (2010) observed an increase in the total soluble solids content of watermelon supplied with higher levels of tithonia manure.

Ahmad *et al*. (2015) also reported a positive effect of *Tithonia diversifolia* on the total soluble solids content of carrots compared to the control. The findings were similar to those reported by Jama *et al.* (2000) who found 4.1%K and 3.5%N (on a dry weight basis) in tithonia. According to Robinson *et al.* (2014). Trichoderma was found to enhance the fruit quality parameters such as ascorbic acid content in grapes. The results were similar to those of Pascale *et al.* (2018), who observed an increase in fruit quality parameters in grapes due to application of *Trichoderma asperellum*. This supports the findings of this study where *Trichoderma asperellum* resulted in higher levels of total soluble solids and ascorbic acid content in fruits.

5.3 Effects of *Tithonia diversifolia* and *Trichoderma asperrellum* on Disease incidence and Severity (*Botrytis cinerea*)

Beneficial microbes can also act as bio control organisms, helping plants defend themselves from attack by pathogens. This is the case for fungi of the genus *Trichoderma*, which are excellent mycoparasites of plant pathogens, directly protecting plants against them (Faruk & Rahman 2015). In addition, *Trichoderma* spp. can also enhance the plant defence system through priming, enabling the plant to respond in a much faster and stronger manner to pathogen attack (Hermosa *et al*., 2012; Malmierca *et al*., 2015). This implies beneficial

microbes can modulate the plant defence system to enable the interaction (Hassani *et al.,* 2018; Jayaraman *et al.,* 2014).

In the present study, *Trichoderma asperellum* at 80mL and *Tithonia diversifolia* at 750mL recorded the lowest disease incidence and severity compared to control in both experiments. It was demonstrated that trichoderma spp. strains can survive in the aerial part of the plants for long periods and can act as antagonists of fungal pathogens in a wide range of crops (Loreto *et al*., 2010; Woo *et al*., 2014). Trichoderma spp. were able to control *Botrytis cinerea* in strawberry because, they can secrete cell wall degrading enzymes like chitinases and volatile and non- volatile compounds which enter the cell in the form of signal and triggers secondary messengers and altering the metabolic pathway of the pathogen (Zeilinger *et al*., 2016). According to Katatny and Emam (2012), trichoderma isolates were able to control Rhizopusspp, Aspergillus, Fusarium, and Alternaria leading to post-harvest rot in tomato. Similar results were observed by Carrero *et al*. (2016) who found trichoderma species to be effective in controlling post-harvest rots on tomato in invitro. A study conducted by Isabella *et al*. (2016) indicated that *Trichoderma asperellum* was effective in controlling. *Solanii* in cucumber seedlings. Several reports indicate the success of Trichoderma isolates in controlling damping-off and root rot caused by Pythium species in different crops (Kipngeno *et al.,* 2015).The Antimicrobial activities could be the result of several secondary metabolites such as peptaibols, terpenes, polyketides, gliotoxin, and gliotoxin produced by fungi (Vinale *et al.,* 2012). Other metabolites include tricholin, harzianic acid, viridian, gliosoprnins, heptelidic acid, 6-pentyl-α-pyrone, and massoilactone, which inhibit phytopathogen fungi growth (Mukherjee *et al*., 2012). The ability of trichoderma spp to suppress, parasitize, and even kill other pathogenic fungi is documented as a key mechanism for its success as biological control (Mukherjee *et al*., 2012)

Fernandez *et al. (*2014) found trichoderma being more effective in controlling *Botrytis cinerea* in the field and after storage. In a research conducted by Martinez *et al.* (2009) *Trichoderma asperellum* inoculum was used as a substrate to protect against *Phytophtora cactorum* and *Verticilium dahliae* in strawberry. According to Pascale *et al.* (2018), trichoderma species were able to control powdery mildew in grapes by inducing the resistance in uninfected leaf tissue locally, systemically through decreasing the reactive oxygen species or enhancing the development of phytohormones. Similar results were reported by Tchameni *et al*. (2011) who observed the ability of *Trichoderma asperellum* in controlling black pod diseases in cacao. The obtained data are in agreement with those

obtained by Segarra *et al*. (2013), who found that *Trichoderma asperellum* strain suppressed zoospore germination of *Phytophthora capsici.*

The use of plant extracts in the control of disease incidences and severity in strawberry is worthwhile attempt to grow healthy plants in the humid tropics where the use of synthetic agrochemicals is not allowed and hazardous to human and the general environment. In the present study combination of *Tithonia diversifolia* and *Trichoderma asperellum* was significant in controlling disease incidence and severity in strawberry fruits. Extracts from various Asteraceae including *Tithonia diversifolia*, were effective inhibitors of the mycelia growth of *F. oxysporium* and *Trichophyton mentagrophytes* (Agboola *et al*., 2016). Enikuomehin (2005) reported that Cercospora leaf spot disease of sesame (*Sesanum indicum L*.) was controlled with plant extracts including extract of *Tithonia diversifolia*. Research by Llondu *et al.* (2014) confirmed that field evaluation *Tithonia diversifolia* extracts were effective in the control of leaf spot disease of sweet potatoes. Barboza *et al.* (2018) reported that leaf extract of *Tithonia diversifolia* consisted of thithoniaquinone A and tithoniamide B, psoralen, and Iquebrachitol. Thithoniaquinone-A and psoralen were strongly fungicidal and antibacterial. These compounds contributed to low *Botrytis cinerea* incidences and severity in strawberry in the present study.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings, it can be concluded that;

i). Use of *Tithonia diversifolia* and *Trichoderma asperellum* influences growth and yield of strawberry fruits. *Tithonia diversifolia* at 750mL and *Trichoderma asperellum* 80mL produced the highest number of leaves and the longest stems in Strawberry. The increase in nutrient uptake plants induced by *Trichoderma asperellum* as biological control is an important factor contributing to plant growth promotion and disease suppressiveness. *Tithonia diversifolia* is a botanical control rich in nutrients such as Nitrogen, Potassium and phosphorous which enhances plant growth.

ii). Use of *Tithonia diversifolia* and *Trichoderma asperellum* influences disease incidence and severity in strawberry fruits. Interaction between *Tithonia diversifolia* and *Trichoderma .asperellum* reduced disease severity by recording the lowest percentage compared to control.

iii). Different concentrations of *Tithonia diversifolia* and *Trichoderma asperellum* significantly influences the quality of strawberry fruits and therefore improving the shelf life.

6.2 Recommendations

Based on the results of this study, the following recommendations can be made;

i) Production of both strawberry can be enhanced by the use of *Tithonia diversifolia* 750mL and *Trichoderma asperellum* 80mL especially in agro-ecological zones similar to the current study area since it promoted total yield and also reduced disease incidence and severity.
ii) Since *Trichoderma asperellum* and *Tithonia diversifolia* reduced disease incidence and severity, more research should be done on improvement of strawberry skin hardness and outward appearance after harvesting so as to improve its shelflife.
iii) Application of *Trichoderma asperellum* and *Tithonia diversifolia* improved Total soluble solids and Ascorbic acid content in strawberry.Strawberry growers are adivised to use *Trichoderma asperellum at* 80mL and *Tithonia diversifolia at* 750mL as it resulted in strawberries with the highest content of total soluble solids and ascorbic acid content

REFERENCES

Ademiluyi, B.O. (2012). Effect of *Tithonia diversifolia* (Hemsl) on the growth and yield of okra (*Abelmuscus esculentus*). *Journal of Agricultural Science and Technology*, 2:219-222

Adeniyan, O. B., Ojeniyi S. O., & Akinola, M. O. (2008). Effect of weed mulch on soil properties, growth tuber yield and nutrient content of Yam (*Dioscorearotundata*poir). *Journal of Agricultural Sciences, 2*(4):70- 74.

Agboola, O. O., Oyedeji, S., Ajao, A., & Aregbesola, O. (2016). Chemical composition and antimicrobial activities of essential oil extracted from *Tithonia diversifolia* (Asteraceae) flower. *Journal of Bioresources and Bioproducts, 1*(4):169-176.

Aghofack-Nguemezi, J., & Dzukam, H. J. C. (2016). Extracts and powders of *Solanum aculeastrum* and *Tithonia diversifolia* induced an improvement of *Solanum lycopersicum* plant growth and development and fruit quality. *International Journal of Biology Research, 1*(5):43-49

Aguyoh, J. N., Audi, W., Saidi, M., & Gao-Qiong, L. (2010). Growth, yield and quality response of watermelon (*Citrullus lanatus* [thunb] mansf. and nakai) cv. crimson sweet) subjected to different levels of tithonia manure. *International Journal of Science and Nature, 1*(1):7-11.

Agyakwa, C. W., and & Akobundu, I. O. (1998). A Hand book of West African Weeds International Institute of Tropical Agriculture. *Ibadan, Nigeria. African Book Builders,* 352.

Ahmad, Z., Ali, N., Ahmad, M., Ulhag, S., & Ahmad, S. (2015). Yield and economics of carrot production in organic farming. *Sarhad Journal of Agriculture,* 21:3-8.

Ajao, A. A., & Moteetee, A. N. (2017). *Tithonia diversifolia* (Hemsl) an invasive plant of significant ethnopharmacological importance: A review. *South African Journal of Botany, 113*: 396-403.

Akanbi, W. B., Adebayo, T. A., Togun, O. A., Adeyeye, A. S., & Olaniran, O. A. (2007). The use of compost extract as foliar spray nutrient source and botanical insecticide in *Telfairiaoccidentalis*. *World Journal of Agricultural Sciences, 3*(5):642-652.

Altieri, M. A., & Anderson, M. K. (1986). An ecological basis for the development of alternative agricultural systems for small farmers in the Third World. *American Journal of Alternative Agriculture, 1*(1):30-38.

Angelini, R. M. D. M., Pollastro, S., & Faretra, F. (2016). Genetics of *Botrytis cinerea*. Botrytis–the Fungus, the Pathogen and its Management in Agricultural Systems. *Journal of Agricultural and Food Chemistry 3*(5):35-53.

Archbold, D. D., Hamilton-Kemp, T. R., Barth, M. M., & Langlois, B. E. (1997). Identifying natural volatile compounds that control gray mold (*Botrytis cinerea*) during postharvest storage of strawberry, blackberry, and grape. *Journal of Agricultural and Food Chemistry,45*(10):4032-4037.

Babajide, P. A., Akanbi, W. B., Olabode, O. S., Olaniyi, J. O., & Ajibola, A. T. (2012). Influence of pre-application handling techniques of *Thitonia diversifolia* Hemsl. A. Gray residues on sesame, in south-western Nigeria. *Journal of Animal and Plant Sciences.* 15:2135-2146.

Bae H. (2011). Trichoderma species as abiotic and biotic stress quenchers in plants. *Research Journal of . Biotechnology. 6* (3):73-79

Banaeian, N., Omid, M., & Ahmadi, H. (2011). Energy and economic analysis of greenhouse strawberry production in Tehran province of Iran. *Energy Conversion And Management*, *52*(2): 1020-1025. https://doi.org/10.1016/j.enconman.2010.08.030

Barboza, B. R., Silva, B. R., Ramos, B., Moura, M. C., Napoleão, T. H., Correia, M. T., & Melo, C. M. L. (2018). Phytochemical bioprospecting, antioxidant, antimicrobial and cytotoxicity activities of saline extract from Tithonia diversifolia (Hemsl) A. Gray leaves. *Asian Pacific Journal of Tropical Biomedicine*, *8*(5), 245. https://doi.org/10.4103/2221-1691.233005

Basu, A., Nguyen, A., Betts, N., & Lyons, T. (2013). Strawberry As a Functional Food: An Evidence-Based Review. *Critical Reviews In Food Science And Nutrition*, *54*(6), 790-806.https://doi.org/10.1080/10408398.2011.608174

Bendahmane, B. S., Mahiout, D., Benzohra, I. E., & Benkada, M. Y. (2012). Antagonism of three Trichoderma species against *Botrytis fabae* and *B. cinerea*, the causal agents of chocolate spot of faba bean (Vicia faba L.) in Algeria. *World Applied Sciences Journal*, *17*(3): 278-283. https://doi.org/10.18805/ag.d-265

Bhutia, D., Zhimo, Y., Kole, R., & Saha, J. (2016). Antifungal activity of plant extracts against Colletotrichum musae, the post harvest anthracnose pathogen of banana cv. Martaman. *Nutrition & Food Science*, *46*(1):2-15. https://doi.org/10.1108/nfs-06-2015-0068

Biljana, G. & Jugoslav, J. (2012) *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. *Applied Technologies Innovations*, 7:67-76. https://doi.org/10.15208/ati.2012.9

Bisen, K., Keswani, C., Mishra, S., Saxena, A., Rakshit, A., & Singh, H. B. (2015). Unrealized potential of seed biopriming for versatile agriculture. In *Nutrient use efficiency:frombasicstoadvances* (pp.193-206). https://doi.org/10.3390/agriculture11040372

Bisen, K., Keswani, C., Patel, J. S., Sarma, B. K., & Singh, H. B. (2016). Trichoderma spp.: Efficient inducers of systemic resistance in plants. *Microbial-mediated Induced Systemic Resistance in Plants*, 185-195. https://doi.org/10.1007/978-981-10-0388-2_12

Carisse, O. (2016). Epidemiology and aerobiology of botrytis spp. *Botrytis the Fungus, the Pathogen and its Management in Agricultural Systems*, 127-148. https://doi.org/10.1007/978-3-319-23371-0_7

Carrero-Carrón, I., Trapero-Casas, J. L., Olivares-García, C., Monte, E., Hermosa, R., & Jiménez-Díaz, R. M. (2016). *Trichoderma asperellum* is effective for biocontrol of Verticillium wilt in olive caused by the defoliating pathotype of *Verticillium dahliae*. *Crop Protection*, *88*:45-52. https://doi.org/10.1016/j.cropro.2016.05.009

Chagas-Paula, D. A., Oliveira, R. B., Rocha, B. A., & Da Costa, F. B. (2012). Ethnobotany, chemistry, and biological activities of the genus Tithonia (Asteraceae). *Chemistry and biodiversity*, *9*(2):210-235. https://doi.org/10.1002/cbdv.201100019

Chen, J. L., Sun, S. Z., Miao, C. P., Wu, K., Chen, Y. W., Xu, L. H., .& Zhao, L. X. (2016). Endophytic Trichoderma gamsii YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of Panax notoginseng. *Journal of Ginseng Research*, *40*(4):315-324. https://doi.org/10.1016/j.jgr.2015.09.006

Chiejina, N. V., & Ukeh, J. A. (2013). Efficacy of *Afromomum melegueta* and *Zingiber officinale* extracts on fungal pathogens of tomato fruit. *IOSR Journal of Pharmacy and Biological Sciences*, *4*(6):13-16.

Choudhury, D., Dobhal, P., Srivastava, S., Saha, S., & Kundu, S. (2018). Role of botanical plant extracts to control plant pathogens-A review. *Indian Journal of Agricultural Research*, *52*(4):341-346.

Contreras-Cornejo, H. A., Macías-Rodríguez, C., Cortés-Penagos, L., & López-Bucio, J. (2009). Trichoderma virens, a plant beneficial fungus, enhances biomass production

and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant Physiology*, 149:1579–1592

Contreras-Cornejo, H. A., Macías-Rodríguez, L., Del-Val, E. K., & Larsen, J. (2016). Ecological functions of Trichoderma spp. and their secondary metabolites in the rhizosphere: interactions with plants. *Microbiology ecology*, *92*(4): 36-40.

Cosseboom, S. D., Ivors, K. L., Schnabel, G., Bryson, P. K., & Holmes, G. J. (2019). Within-season shift in fungicide resistance profiles of Botrytis cinerea in California strawberry fields. *Plant disease*, *103*(1): 59-64.

Costa, L. B., Rangel, D. E., Morandi, M. A., & Bettiol, W. (2013). Effects of UV-B radiation on the antagonistic ability of Clonostachysrosea to *Botrytis cinerea* on strawberry leaves. *Biological control*, *65*(1): 95-100.

Dahunsi, O. S., Oranusi, S., & Efeovbokhan, E. V. (2017). Anaerobic mono-digestion of *Tithonia diversifolia* (Wild Mexican sunflower). *Energy Conversion and Management*, *148*: 128-145.

Davis, T. M. (2015). Geographical distribution of strawberries. University of New Hampshire New Hampshire Agricultural Experiment Station, Durham, NH.

De Corato, U., Salimbeni, R., De Pretis, A., Avella, N., & Patruno, G. (2017). Antifungal activity of crude extracts from brown and red seaweeds by a supercritical carbon dioxide technique against fruit postharvest fungal diseases. *Postharvest Biology and Technology*, *131*: 16-30.

Deberdt, P., Mfegue, C. V., Tondje, P. R., Bon, M. C., Ducamp, M., Hurard, C., & Cilas, C. (2008). Impact of environmental factors, chemical fungicide and biological control on cacao pod production dynamics and black pod disease (Phytophthora megakarya) in Cameroon. *Biological Control*, *44*(2): 149-159.

Droby, S., & Lichter, A. (2014). Post-harvest Botrytis infection: Etiology, development and management. Botrytis: *Biology, pathology and control* (pp. 349–367).

El_Komy, M. H., Saleh, A. A., Eranthodi, A., & Molan, Y. Y. (2015). Characterization of novel Trichoderma asperellum isolates to select effective biocontrol agents against tomato Fusarium wilt. *The Plant Pathology Journal*, *31*(1): 50.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*, *19*(10):709-714.

Elad, Y., & Shtienberg, D. (1994). Effect of compost water extracts on grey mould *(Botrytis cinerea*).*Crop protection*, *13*(2):109-114.

Elad, Y., Vivier, M., & Fillinger, S. (2016). Botrytis, the good, the bad and the ugly. In *Botrytis–The fungus, the pathogen and its management in agricultural systems* (pp. 1-15). Springer, Cham.

Elad, Y., Williamson, B., Tudzynski, P., & Delen, N. (2007). *Botrytis spp*. and diseases they cause in agricultural systems–an introduction. *Biology, Pathology and Control* (pp. 1-8).

Elshahawy, I. E., & El-Mohamedy, R. S. (2019). Biological control of Pythium damping-off and root-rot diseases of tomato using Trichoderma isolates employed alone or in combination. *Journal of Plant Pathology*, *101*(3):597-608.

Etalo, D. W., Jeon, J. S., & Raaijmakers, J. M. (2018). Modulation of plant chemistry by beneficial root microbiota. *Natural product reports*, *35*(5):398-409.

Fahrurrozi, k., Sariasih, Y., Muktamar, Z., Setyowati, N., Chozin, M. & Sudjatmiko, S. (2017). Identification of nutrient contents in six potential green biomasses for developing liquid organic fertilizer in closed agricultural production system. *International Journal on Advanced Science Engineering Information Technology*.7:2088-5334.

FAO STAT. (2014). Food and Agriculture Organization of the United Nations. FAOSTAT Database. Rome, Italy: FAO. Retrieved October 9, 2017 from http:// Faostat3.Fao.Org/Home/E.

FAOSTAT. (2018).Strawberry world production Retrived from http:www.fao.org/faostat/en/#data/QC.

Faruk, M., & Rahman, M. (2015). Efficacy of carrier materials to formulate *Trichoderma harzianum* bio-fungicide in controlling seedling disease (Rhizoctonia solani) of eggplant. *Journal of Global Agriculture and Ecology*, *2*(1): 17-25.

Fernández, E., Segarra, G., & Trillas, M. I. (2014). Physiological effects of the induction of resistance by compost or Trichoderma asperellum strain T34 against *Botrytis cinerea* in tomato. *Biological Control*, 78:77-85.

Fillinger, S., & Elad, Y. (Eds.). (2016). *Botrytis-the fungus, the pathogen and its management in agricultural systems*. Switzerland: Springer International Publishing, (pp. 189-216)

Fontenelle, A. D. B., Guzzo, S. D., Lucon, C. M. M., & Harakava, R. (2011). Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by Trichoderma spp. *Crop Protection*, *30*(11): 1492-1500.

Forbes-Hernandez, T. Y., Gasparrini, M., Afrin, S., Bompadre, S., Mezzetti, B., Quiles, J. L., & Battino, M. (2016). The healthy effects of strawberry polyphenols: which strategy behind antioxidant capacity. *Critical reviews in food science and nutrition*, *56*(1): 46-59.

Genre, A., & Russo, G. (2016). Does a common pathway transduce symbiotic signals in plant–microbe interactions. *Frontiers in plant science*, *7*:96 -102.

Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., & Battino, M. (2015). Strawberry as a health promoter: an evidence based review. *Food and function*, *6*(5): 1386-1398.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: composition, nutritional quality, and impact on human health. *Nutrition*, *28*(1):9-19.

Gonçalves, A. H., Chagas, L. F., dos Santos, G. R., Fidelis, R. R., Magno Filho, R. C., Miller, L. O., & Junior, A. F. C. (2018). Trichoderma efficiency in the maintenance and productivity of soybean plants in producing savanna regions, Tocantins, Brazil. *Revista de Ciências Agrárias*, *41*(1):175-181.

Guetsky, R., Shtienberg, D., Elad, Y., & Dinoor, A. (2010). Combining biocontrol agents to reduce the variability of biological control. *Phytopathology*,*91*(7), 621-627.

GuongA, V. T., HienA, N. X., & MinhB, D. (2010). Effect of fresh and composted organic amendment on soil compaction and soil biochemical properties of citrus orchards in the Mekong Delta, Vietnam. In *19th World soil congress, Brisbane, Australia*.

Hafifah, Sudiarso, Maghfoer, M. D. & Prasetya, B. (2016). The potential of *Tithonia diversifolia* green manure for improving soil quality for cauliflower (Brassica oleracea var. Brotrytis L.). *Journal of Degraded and Mining Lands Management*. 3:499-50

Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. *Journal of chemical biology*, *7*(4): 133-141.

Haidar, R., Fermaud, M., Calvo-Garrido, C., Roudet, J., & Deschamps, A. (2016). Modes of action for biological control of *Botrytis cinerea* by antagonistic bacteria. *Phytopathologia Mediterranea*, (pp301-322).

Hajieghrari, B., & Mohammadi, M. (2016). Growth-promoting activity of indigenous Trichoderma isolates on wheat seed germination, seedling growth and yield. *Australian Journal of Crop Science*, *10*(9):1339-1347.

Häkkinen, S. H., & Törrönen, A. R. (2000). Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food Research International*, *33*(6):517-524.

Hancock, J. F., Lavín, A. R., & Retamales, J. B. (2013). Our southern strawberry heritage: *Fragariachiloensis* of Chile. *HortScience*, *34*(5): 814-816.

Hanounik, S. B., & Robertson, L. D. (1988). New sources of resistance in Vicia faba to chocolate spot caused by *Botrytis fabae*. *Plant Disease*, *72*(8):696-698.

Haque, M. M., Ilias, G. N. M., & Molla, A. H. (2012). Impact of Trichoderma-enriched biofertilizer on the growth and yield of mustard (Brassica rapa L.) and tomato (Solanum lycopersicon Mill.). *The Agriculturists*, *10* (2):109-119.

Hasan, Z. A. E., Mohd Zainudin, N. A. I., Aris, A., Ibrahim, M. H., & Yusof, M. T. (2020). Biocontrol efficacy of Trichoderma asperellum-enriched coconut fibre against Fusarium wilts of cherry tomato. *Journal of applied microbiology*, *129* (4):991-1003.

Hassani, M. A., Durán, P., & Hacquard, S. (2018). Microbial interactions within the plant holobiont. *Microbiome, 6* (1):58.

HCD (Horticultural Crops Directorate). (2014). *Horticulture Validated Report*. Fruits and Vegetables. Agricultural Information Resource Centre, Nairobi, Kenya.

HCDA (Horticultural Crops Development Authority). (2006). *Horticulture Validated Report*. Fruits and Vegetables. Agricultural Information Resource Centre, Nairobi, Kenya.

Hermosa, R., Viterbo, A., Chet, I., & Monte, E., (2012). Plant-beneficial effects of Trichoderma and of its genes. Microbiology 158: 17-25.

Herrera-Parra, E., Cristóbal-Alejo, J., & Ramos-Zapata, J. A. (2017). Trichoderma strains as growth promoters in *Capsicum annuum* and as biocontrol agents in *Meloidogyne incognita*. *Chilean journal of agricultural research*, *77*(4): 318-324.

Huang, L. Q., Niu, Y. C., Su, L., Deng, H., & Lyu, H. (2020). The potential of endophytic fungi isolated from cucurbit plants for biocontrol of soilborne fungal diseases of cucumber. *Microbiological research*, *231*:126-369.

Hussain M. A., T. Mukhtar & M. Z. Kayani. (2011). Efficacy evaluation of *Azadirachta indica, Calotropis procera, Datura stramonium* and *Tagetes erecta* against root-knot nematodes Meloidogyne incognita. *Pakistan Journal of Botany,*43*:197–204*

Ilondu, E. M., Ojeifo, I. M., & Egho, E. O. (2014). Field evaluation of leaf extract of some Asteraceae for the management of leafspot disease of sweet potatoes in Abraka, Delta State,Nigeria. *Academia Journal of Agricultural Research*, *2*(3):80-86.

Ippolito, A., & Nigro, F. (2016). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection*, 19:715-723

Jacometti, M. A., Wratten, S. D., & Walter, M. (2010). Alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. *Australian Journal of Grape and Wine Research*, *16*(1):154-172.

Jaetzold, R., H. Schmidt, B. Hornetz, & Shisanya, C. (2006) "Ministry of Agriculture Farm Management Handbook of Kenya VOL. II-Part C Subpart C1." *Nairobi, Kenya: Ministry of Agriculture*.

Jamal, B., Palm, C. A., Buresh, R. J., Niang, A., Gachengo, C., Nziguheba, G., & Amadalo, B. (2000). *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya: a review. *Agroforestry Systems*, *49*(2):201-221.

Janisiewiez, W. J. (1998). Biocontrol of postharvest diseases of temperate fruits - challenges and opportunities In: Boland GJ and Kuykendall LD (eds) *Plant-Microbe Interactions and Biological Control.* (pp. 171-198)

Jayaraman, S., & Mohamed, R. (2015). Crude extract of Trichoderma elicits agarwood substances in cell suspensionculture of the tropical tree, Aquilaria malaccensis Lam. *Turkish Journal of Agriculture and Forestry*, 39(2):163-173.

Jayasundara, J. M. N. P., Jayasekara, R., & Ratnayake, R. M. C. S. (2016). Liquid organic fertilizers for growth enhancement of *Abelmoschus esculentus* (L.) Moench and Alternanthera sessilis (L.) DC. *Tropical Plant Research*, *3*(2):334-340.

Jimoh, M., Enikuomehin, O. A., Afolabi, C. G., Olowe, V. I. O., & Egbontan, A. (2016). Achieving improved control of foliar diseases of sesame (Sesamum indicum L.) intercropped with maize (Zea mays L.) through foliar spray of plant extracts. *Archives of Phytopathology and Plant Protection*, *49*(19):586-600.

Jiri, O., & Waddington, S. R. (1998). Leaf prunings from two species of tithonia raise maize grain yield in Zimbabwe, Newsletter of Soil Fertility Net, Harare, Zimbabwe. *Target*, *16*(3) :4-5.

John-Dewole, O. O., & Oni, S. O. (2013). Phytochemical and antimicrobial studies of extracts from the leaves of Tithonia diversifolia for pharmaceutical importance. *International journal of Pharma and Bio Science*, *6*, (2):21-25.

Kamboj, A., & Saluja, A. (2008). Ageratum conyzoides L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy*, *2*(2):59.

Kandungu, J., Anjarwalla, P., Mwaura, L., Ofori, D. A., Jammadass, R., Stevenson, P. C., & Smith, P. (2013). Pesticidal Plant Leaflet. *Tithonya diversifolia (Hemsley) A. Gray. Kew Royal Botanic Gardens, World Agroforestry Centre,12* (24):34-45

Kareru, P. G., Keriko, J. M., Kenji, G. M., Thiong'o, G. T., Gachanja, A. N., & Mukiira, H. N. (2010). Antimicrobial activities preparations from plant extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*(3): 56-78

Kasperbauer, M. J. (2010). Strawberry yield over red versus black plastic mulch. *Journal of Crop Science*, *40*(1):171-174.

Katatny, M. H & Emam, A. S. (2020). Control of postharvest tomato rot by spore suspension and antifungal metabolites of *Trichoderma harzianum*. *Journal of Microbiology, Biotechnology and Food Sciences, 9*(5): 1505-1528.

KATC (Kasisi Agricultural Training Centre) & SCC (Swedish Cooperative Centre) (2007). Sustainable agriculture; study circle material. Aquila limited, Lusaka, Zambia.

Kerroum, F., Noureddine, K., Eddine, H. J., & Mebrouk, K. (2015). Biological control of Fusarium crown and root rot disease of tomato by Trichoderma harzianum in the west of Algeria. *International Journal of Science and Nature 6*(3):141-146.

Keswani, C., Mishra, S., Sarma, B.K., Singh, S.P., & Singh, H.B., 2014. Unraveling the efficient applications of secondary metabolites of various Trichoderma spp. Applied. Microbiol and Biotechnology. 98: 533-44.

Khalili, E., Sadravi, M., Naeimi, S., & Khosravi, V. (2012). Biological control of rice brown spot with native isolates of three Trichoderma species. *Brazilian Journal of Microbiology*, *43*(1):297-305.

Kim, J. O., Shin, J. H., Gumilang, A., Chung, K., Choi, K. Y., & Kim, K. S. (2016). Effectiveness of different classes of fungicides on *Botrytis cinerea* causing gray mold on fruit and vegetables. *The Plant Pathology Journal*, *32*(6): 570.

Kim, T. G., & Knudsen, G. R. (2013). Relationship between the biocontrol fungus *Trichoderma harzianum* and the *phytopathogenic* fungus *Fusarium solani Applied Soil Ecology*, *68*:57-60.

Kipngeno, P., Losenge, T., Maina, N., Kahangi, E., & Juma, P. (2015). Efficacy of *Bacillus subtilis* and *Trichoderma asperellum* against *Pythium aphanidermatum* in tomatoes. *Biological Control, 90* (15):92-95

Klopotek, Y., Otto, K., & Böhm, V. (2015). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 53*(14): 5640-5646.

Konstantinou, S., Veloukas, T., Leroch, M., Menexes, G., Hahn, M., & Karaoglanidis, G. (2015). Population structure, fungicide resistance profile, and sdhB mutation frequency of *Botrytis cinerea* from strawberry and greenhouse-grown tomato in Greece. *Plant Disease*, *99*(2):240-248.

Kowalska, J. (2011). Effects of *Trichoderma asperellum* on *Botrytis cinerea*, growth and yield of organic strawberry. *Acta Scientiarum Polonorum Seria HortorumCultus, 10*(4):107-114.

Lei, Z. H. A. O., & Zhang, Y. Q. (2015). Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. *Journal of Integrative Agriculture*, *14*(8):1588-1597.

Li, H., Li, T., Gordon, R. J., Asiedu, S. K., & Hu, K. (2010). Strawberry plant fruiting efficiency and its correlation with solar irradiance, temperature and reflectance water index variation. *Environmental and Experimental Botany*, *68*(2): 165-174.

Liasu, M. O., Atayese, M. O., & Osonubi, O. (2006). Effect of mycorrhiza and pruning regimes on seasonality of hedgerow tree mulch contribution to alley-cropped cassava in Ibadan, Nigeria. *African Journal of Biotechnology*, *14*(5):1341-1349

Lima, G., Ippolito, A., Nigro, F., & Salerno, M. (2007). Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Postharvest Biology and Technology, 10:169-178

Lin, Y. R., Lo, C. T., Liu, S. Y., & Peng, K. C. (2012). Involvement of pachybasin and emodin in self-regulation of *Trichoderma harzianum* mycoparasitic coiling. *Journal of Agricultural and Food chemistry*, *60*(9):2123-2128.

Linthoingambi, W., & Singh, M. S. (2013). Antimicrobial activities of different solvent extracts of *Tithonia diversifolia* (Hemsely) A. Gray. *Asian Journal of Plant Science and Research*, *3*(5):50-54.

López-Bucio, J., Pelagio-Flores, R., & Herrera-Estrella, A. (2015). Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. *Scientia horticulturae*, 196: 109-123.

Loreto, F., & Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs. *Trends in Plant Science, 15*(3): 154-166.

Maas, J. L., Hartung, J. S. Gouin-Behe, C., & Hokanson, S. C. (2000). Sources of resistance for two differentially pathogenic strains of *Xanthomonas fragariae* in *Fragaria* genotypes. *HortScience, 35(*1):128-131.

Mahmood, T., Anwar, F., Abbas, M., & Saari, N. (2012). Effect of maturity on phenolics (phenolic acids and flavonoids) profile of strawberry cultivars and mulberry species from Pakistan. *International Journal of Molecular Sciences*, *13*(4):4591-4607.

Majidi, H., Minaei, S., Almasi, M., & Mostofi, Y. (2011). Total Soluble Solids, Titratable Acidity and ripening index of Tomato in Various Storage Conditions. *Basic and Applied Sciences* 5(12):1723-1726pp.

Malmierca, M. G., McCormick, S. P., Cardoza, R. E., Monte, E., Alexander, N. J., & Gutiérrez, S. (2015). Trichodiene production in a *Trichoderma harzianum* erg1-silenced strain provides evidence of the importance of the sterol biosynthetic pathway in inducing plant defense-related gene expression. *Molecular Plant-Microbe Interactions*, *28*(11):1181-1197.

Marinova, D., & Ribarova, F. (2007). HPLC determination of carotenoids in Bulgarian berries. *Journal of Food Composition and analysis*, *20*(5): 370-374.

Martin, E., Tepe, E., & Wold-Burkness, S. (2014). Cold climate strawberry farming.

Martínez-Ferri, E., Soria, C., Ariza, M. T., Medina, J. J., Miranda, L., Domíguez, P., & Muriel, J. L. (2016). Water relations, growth and physiological response of seven strawberry cultivars (*Fragaria× ananassa Duch*.) to different water availability. *Agricultural Water Management*, *164*:73-82.

Martínez-Medina, A., Pascual, J. A., Lloret, E. & Roldan, A. (2009). Interactions between arbuscularmycorrhizal fungi and *Trichoderma harzianum* and their effects on Fusarium wilt in melon plants grown in seedling nurseries. *Journal of the Science of Food and Agriculture*, *89*(11): 1843-1850.

Mastouri, F. (2010). Use of Trichoderma spp. to improve plant performance under abiotic stress. PhD thesis, Cornell University, USA.

Mazhabi, M., Nemati, H., Rouhani, H., Tehranifar, A., Mahdikhani-Moghadam, E., & Kaveh, H. (2011). How may Trichoderma application affect vegetative and qualitative traits in tulip "Darwin hybride" cultivar. *Journal of Biology and Environmental Science* ,*5*(3): 177-182.

Mertely, J. C., Seijo, T. E., MacKenzie, S. J., Moyer, C., & Peres, N. A. (2019). Effect of timing of preharvest fungicide applications on postharvest Botrytis fruit rot of annual strawberries in Florida. *Plant Health Pr*ogress , *10*(5): 1-6.

Meunchang, S., Panichsakpatana, S., & Weaver R .W. (2006). Tomato growth in soil amended with sugar mill by products compost. *Plant and Soil*,280 (1-2):171–176.

Ming, L. C. (1999). *Ageratum conyzoides*: A tropical source of medicinal and agricultural products. *Perspectives on new crops and new uses*, 469-473.

Mohamed, N., Lherminier J., Farmer M. J., Fromentin J., Béno N., Houot V., Milat M. L., & Blein J. P. (2007). Defense responses in grapevine leaves against Botrytis cinerea induced by application of a *Pythiumoligandrum* strain or its elicitin, oligandrin, to roots. *Phytopathology, 97* (5): 611–620.

Molla, A.H., Fakhrul Razi A., & Hanafi M.M. (2005). Compost produced by solid state bioconversion of biosolids: a potential source for plant growth and environmental friendly disposal. *Communications in Soil Science and Plant Analysis, 36*(11):1435–1447.

Mukherjee, P. K., Buensanteai, N., Morán-Diez, M .E., Druzhinina I., & Kenerley CM (2012). Functional analysis of non-ribosomal peptide synthetases (NRPSs) in Trichoderma virens reveals a polyketide synthase (PKS)/NRPS hybrid enzyme involved in the induced systemic resistance response in maize. Microbiology. 155-165

Muktamar, Z., Sudjatmiko, S., Fahrurrozi, F., Setyowati, N., & Chozin, M. (2017). Soil chemical improvement under application of liquid organic fertilizer in closed agriculture system. *Journal of Agricultural Technology*, 13:1715-1727

Murray, S.l., Thomson, C., Chini, A., Read, N. D., & Loake G. J .(2002) Characterization of a Novel, Defense-Related *Arabidopsis*, Isolated by *Luciferase Imagin*. *Molecular Plant—Microbe Interactions*, *15* (6):57-566.

Mustonen, P. S. J., Oelberman, M., & Kass, D. C. (2012). Using Tithonia diversifolia (Hemsl.) Gray in a short fallow system to increase soil phosphorus availability on a Costa Rican Andosol. *Journal of Agricultural Science*, *4*(2): 91.

Mwangi, P.M., & Mathenge, P.W. (2014). Comparison of tithonia (*Tithonia diversifolia*) green manure, poultry manure and inorganic sources of nitrogen in the growth of kales (Brassicae oleraceae) in Nyeri County, Kenya. *African Journal of Food Agriculture, Nutrition and Development*.14:8791-8808

Myresiotis, C. K., Karaoglanidis, G. S., & Tzavella-Klonari, K. (2007). Resistance of *Botrytis cinerea* isolates from vegetable crops to anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole, and dicarboximide fungicides. *Plant Disease*, *91*(4): 407-413.

Naher, L., Yusuf, U. K., Ismail, A., & Hossain, K. (2014). Trichoderma spp.: a biocontrol agent for sustainable management of plant diseases. *Pakistan Journal of Botany*, *46*(4):1489-1493.

Neri, D., Lodolini, E. M., Savini, G., Sabbatini, P., Bonanomi, G., & Zucconi, F. (2002). Foliar application of humic acids on strawberry (cv Onda*). International Society of Horticultural Sciences*, *23*(12):297-302.

Nicot, P. C., Stewart, A., Bardin, M., & Elad, Y. (2016). Biological control and biopesticide suppression of Botrytis-incited diseases. In *Botrytis–the fungus, the pathogen and its management in agricultural systems* (pp. 165-187).

Njenga, J. K. (2019). Efficacy of Neem, Tithonia And Tephrosia Leaf Extracts in Management of Root-Knot Nematodes in French Beans *(Phaseolus Vulgaris L.)* in Tharaka Nithi County, Kenya (Doctoral dissertation, Chuka University).

Obua, B. E., McAlbert, F. U., Okoro, B. O., & Efrenie, S. (2012). Survey of the diversity of forage Plants used in feeding Pigs in smallholder farms in Southeastern Nigeria. *International Journal of Agriculture and Rural Development*, *15*(3): 1310-1316.

Ogbebor, N. O., Adekunle, A. T., & Enobakhare, D. A. (2007). Inhibition of *Colletotrichumg loeosporioides* (Penz) causal organism of rubber (Heveabrasiliensis Muell. Arg.) Leaf spot using plant extracts. *African Journal of Biotechnology*, *6*(3),:57-65.

Ogunfolakan, O., Kolawole, O. S., & Olowe, A. O. (2010). In vitro antimicrobial activity of *Tithonia diversifolia* leaf extracts on bacterial isolates from wound infections from a Nigerian hospital. *Research Journal of Medical Sciences*, *4*(5):305-308.

Olabode, O. S., Sola, O., Akanbi, W. B., Adesina, G. O., & Babajide, P. A. (2007). Evaluation of *Tithonia diversifolia* (Hemsl.) A Gray for soil improvement *World Journal of Agricultural Sciences, 3*(4):503-507.

Ommati, F., & Zaker, M. (2012). Evaluation of some Trichoderma isolates for biological control of potato wilt disease (*Fusarium oxysporum*) under laboratory and greenhouse conditions. *Journal of Crop Protection, 1*(4):279-286.

Omokhua, A. G., Abdalla, M. A., Van Staden, J., & McGaw, L. J. (2018). A comprehensive study of the potential phytomedicinal use and toxicity of invasive Tithonia species in South Africa. *BMC complementary and alternative medicine*, *18*(1):1-15.

Opala P.A., Kisinyo, P.O & Nyambati, R.O (2015).Effects of *Tithonia diversifolia* farmyard manure and urea, and phosphate fertilizer application methods on maize yields in western Kenya. *Journal of agriculture and rural Development in the tropics and subtropics 116*(1):1-9.

Pascale, A., Vinale, F., Manganiello, G., Nigro, M., Lanzuise, S., Ruocco, M., & Lorito, M. (2017). Trichoderma and its secondary metabolites improve yield and quality of grapes. *Crop protection*, *92*:176-181.

Pena, C. D., Bartolome, L.D. I., & Banwa, T.P. (2013).The potential of *Tithonia diversifolia* (Wild sunflower) as organic folia fertilizer .*European Scientific journal Special Edition*.465-468.

Perelló, A. E., Moreno, M. V., Mónaco, C., Simón, M. R., & Cordo, C. (2009). Biological control of Septoriatritici blotch on wheat by Trichoderma spp. under field conditions in Argentina.*Journal of BioControl*, *54*(1):113-122.

Petrasch, S., Knapp, S. J., Van Kan, J. A., & Blanco-Ulate, B. (2019). Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. *Molecular Plant Pathology*, *20*(6):877-892.

Phatik, T., Das, J., & Boruah, P. (2014). Antifungal activity of *Polygonum hydropiper* and *Solanum melongena* against plant pathogenic fungi. *Plant Archives*, *14*(1):15-17.

Pill, W.G., Collins, C.M., Goldberger, B., & Gregory, N., (2009). Responses of non-primed or primed seeds of 'Marketmore 76'cucumber (*Cucumis sativus L*.) slurry coated with Trichoderma species to planting in growth media infested with Pythium aphanidermatum. *Science Horticultural-Amsterdam*. 121: 54-62.

Qualhato, T.F., Lopes, A.S., Steindorff, R.S., Branda, R. S. A., & Ulhoa C .J. (2013) Mycoparasitism studies of Trichoderma species against three phytopathogenic fungi: Evaluation of antagonism and hydrolytic enzyme production. *Biotechnology Letters*., 35:1461-1468

Rashidi, M., & Keshavarzpour, F. (2007) Effect of different tillage methods on soil physical properties and crop yield of watermelon (Citrullus vulgaris). *ARPN Journal of Agricultural and Biological Science, 2*(6):1-6.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture*, *65*(1):1-24.

Rubinstein, J. (2015*). Fragaria xananassa*: Past, Present and Future Production of the Modern Strawberry, *World Journal of Agricultural Sciences 12*(10)*:*20-25

Rupp, S., Weber, R. W., Rieger, D., Detzel, P., & Hahn, M. (2017). Spread of *Botrytis cinerea* strains with multiple fungicide resistance in German horticulture. *Frontiers in microbiology*, *7*:20-75.

Saba, H., Vibhash, D., Manisha, M., Prashant, K. S., Farhan, H., & Tauseef, A. (2012). Trichodermaa promising plant growth stimulator and biocontrol agent. *Mycosphere*, *3*(4): 524-531.

Sallam, N., Abo, E., & Hassan, M. (2008). Evaluation of Trichoderma Species as Biocontrol Agents for Damping-Off and Wilt Diseases of *Phaseolus vulgaris L*. and Efficacy of Suggested Formula. *Egyptian Journal of Phytopathology*, *36*(2):81–93

Savini, G., & Neri, D. (2003, October). Strawberry architectural model. In *Euro Berry Symposium-COST-Action 836 Final Workshop* 649 (pp. 169-176).

Scala, F., Raio, A., Zoina, A., Lorito, M., Chincholkar, S., & Mukerji, K. (2007). Biological control of fruit and vegetable diseases with fungal and bacterial antagonists: Trichoderma and Agrobacterium. *Biological control of plant diseases*, 6, 151-178.

Schuster, A., and Schmoll, M. (2010). Biology and biotechnology of Trichoderma. *Applied Microbiology and Biotechnology*, *87*(3):787-799

Segarra, G., Avilés, M., Casanova, E., Borrero, C., & Trillas, I. (2013). Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34. *Phytopathologia Mediterranea*, *35*(25):77-83.

Senhaji, B., Chebli, B., Mayad, E. H., & Ferji, Z. (2014). Antifungal activity of medicinal plants extracts against *Botrytis cinerea* the causal agent of gray mold on tomato. *Journal of Biology, Agriculture and Healthcare*, *4*(26):141-148.

Şesan, T. E., Enache, E., Iacomi, B. M., Oprea, M., Oancea, F., & Iacomi, C. (2015). Antifungal activity of some plant extracts against *Botrytis cinerea* Pers. in the blackcurrant crop (*Ribes nigrum L*.).*Acta Scientiarum. Polonorum., Hortorum Cultus,14*(1):29-43.

Setyowati, N., Sudjatmiko, S., Muktamar, Z., Fahrurrozi, F., Chozin, M., & Simatupang, P. (2018). Growth and yield responses of cauliflower on tithonia (*Tithonia diversifolia*) compost under organic farming practices. *International Journal of Agricultural Technology*, *14*(7): 1905-1914.

Shackleton, R. T., Nunda, W., Beale, T., Van Wilgen, B. W., & Witt, A. B. (2019). Distribution of invasive alien Tithonia (Asteraceae) species in eastern and southern Africa and the socioecological impacts of *T. diversifolia* in Zambia. *Bothalia-African Biodiversity and Conservation*, *49*(1): 1-11.

Sharma, R. R., Krishna, H., Patel, V. B., Dahuja, A., & Singh, R. (2006). Fruit calcium content and lipoxygenase activity in relation to albinism disorder in strawberry. *Scientia Horticulturae*, *97*(2): 150-154.

Shoresh.M, Harman G. E, & Mastouri F. (2010) Induced systemic resistance and plant responses to fungal biocontrol agents.*Annu. Rev Phytopathol*. 48:21-43

Singh, A., Jain, A., Sarma, B.K., Upadhyay, R. S., & Singh, H.B. (2014). Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during *Sclerotium rolfsii* infection. Microbiol. Res. 169: 353-360.

Stringlis, I. A., Yua, K., Feussner, K., Jonge, R., Van Bentum, S., Van Verk, M. C., & Pieterse, C. M. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences*, *115*(22):E5213-E5222.

Sylla, J., Alsanius, B. W., Krüger, E., & Wohanka, W. (2015). Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. *European journal of plant pathology*, *143*(3): 461-471.

Tagne, A. M., Marino, F., & Cosentino, M. (2018). *Tithonia diversifolia* (Hemsl.) A. Gray as a medicinal plant: A comprehensive review of its ethnopharmacology, phytochemistry, pharmacotoxicology and clinical relevance. *Journal of Ethnopharmacology*, *220*:94-116.

Tchameni, S. N., Ngonkeu, M. E. L., Begoude, B. A. D., Nana, L. W., Fokom, R., Owona, A. D., & Kuaté, J. (2011). Effect of *Trichoderma asperellum* and *arbuscular mycorrhizal* fungi on cacao growth and resistance against black pod disease. *Crop protection*, *30*(10): 1321-1327.

Tjamos, E.C., Tjamos, S .E., & Asntoniou, P.P. (2010). Biological management of plant diseases: Highlights on research and application of biological management. *Journal of Plant Pathology*, 92:17-22

Tona, L., Kambu, K., Ngimbi, N., Cimanga, K., & Vlietinck, A. J. (2014). Antiamoebic and phytochemical screening of some Congolese medicinal plants. *Journal of Ethnopharmacology*, *61*(1), 57-65.

Tondje, P. R., Roberts, D. P., Bon, M. C., Widmer, T., Samuels, G. J., Ismaiel, A., & Bateman, R. (2007). Isolation and identification of mycoparasitic isolates of *Trichoderma asperellum* with potential for suppression of black pod disease of cacao in Cameroon. *Biological control*, *43*(2):202-212.

Uddin, A. F. M. J., Hussain, M. S., Rahman, S. K. S., Ahmad, H., & Roni, M. Z. K. (2015). Effect of Trichoderma concentration on the growth and yield of tomato. *Bangladesh Research Publications Journal*, *11*(3): 228-232.

Vauzour, D., Vafeiadou, K., Rendeiro, C., Corona, G., & Spencer, J. P., (2010). The inhibitory effects of berry-derived flavonoids against neurodegenerative processes. *Journal of Berry Research*, *1*(1): 45-52.

Vinale, F., Sivasithamparam K., & Ghisalberti L. (2012). Trichoderma secondary metabolites that affect plant metabolism. *Natural Product Communications*. 7:1545-1550

Walker, A. S., Micoud, A., Rémuson, F., Grosman, J., Gredt, M., & Leroux, P. (2013). French vineyards provide information that opens ways for effective resistance management of *Botrytis cinerea* (grey mould). *Pest Management Science*, *69*(6): 667-678.

Wedge, D. E., Curry, K. J., Kreiser, B., Curry, A., Abril, M., & Smith, B. J. (2013). Fungicide resistance profiles for 13 *Botrytis cinerea* isolates from strawberry in southeastern Louisiana. *International Journal of Fruit Science*, *13*(4): 413-429.

Williamson, B., Tudzynski, B., Tudzynski, P., & Van Kan, J. A. (2007). *Botrytis cinerea*: the cause of Grey mould disease. *Molecular Plant Pathology*, *8*(5): 561-580.

Woo, S. L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N., & Lorito, M. (2014). Trichoderma-based products and their widespread use in agriculture. *The Open Mycology Journal*, *8*(1): 15-21.

Xiaoxue, Y., Hua, C., Jinzhu, S., & Junzheng, Z. (2013). Heterologous expression of an aspartic protease gene from biocontrol fungus *Trichoderma asperellum* in *Pichiapastoris*. *World Journal of Microbiology and Biotechnology*, 13:1373-1376.

Zaghloul, R. A., Neweigy, N. A., Hanafy, E. A., & Khalifa, N. A. (2017). Effectiveness of bio-control agents against tomato soil borne pathogens. In *3rd Environment Conference, Faculty of Science, Zagazig University* (pp. 123-142).

Zeilinger, S., Gruber, S., Bansal, R., & Mukherjee, P. K. (2016). Secondary metabolism in Trichoderma–chemistry meets genomics. *Fungal biology reviews*, *30*(2):74-90.

Zhang, F., Zhu, Z., Yang, X., Ran, W., & Shen, Q. (2013). *Trichoderma harzianum* T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. *Applied Soil Ecology*, *72*:41-48.

APPENDICES

i). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on plant height at 28 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	22.07			
Block	2	0.7429	O.37	1.06	
Ta	2	0.2112	0.10	0.30	0.742
Td	3	8.5035	2.83	8.11	<.001
Ta.Td	6	4.9288	0.82	2.35	0.066
Error	22	7.6904	0.34		
Coefficient of variation	9.7				

ii). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on plant height at 42 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	53.28			
Block	2	1.44	0.72	9.20	
Ta	2	3.51	1.75	22.41	<.001
Td	3	44.30	14.76	188.56	<.001
Ta.Td	6	2.3	0.38	4.90	<.003
Error	22	1.72	0.07		
Coefficient of variation	2.87				

iii). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on plant height at 56 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	27.40			
Block	2	0.85	0.42	6.62	
Ta	2	2.22	1.11	17.19	<.001
Td	3	20.45	6.82	105.41	<.001
Ta.Td	6	2.44	0.40	6.26	<.001
Error	22	1.42	0.06		
Coefficient of variation	1.9				

iv). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on plant height at 70 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	29.32			
Block	2	0.09	0.04	0.29	
Ta	2	11.37	5.68	36.22	<.001
Td	3	10.50	3.50	22.29	<.001
Ta.Td	6	3.89	0.64	4.14	<.006
Error	22	3.45	0.15		
Coefficient of variation	2.4				

v). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on plant height at 84 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	268.79			
Block	2	0.22	0.11	0.40	
Ta	2	18.46	9.23	32.71	<.001
Td	3	228.93	76.31	270.40	<.001
Ta.Td	6	14.95	2.49	8.83	<.001
Error	22	6.20	0.28		
Coefficient of variation	2.6				

vi). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of leaves per plant at 28 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
TOTAL	35	16.27			
Block	2	0.01	0.02	0.02	
Ta	2	1.24	0.62	5.84	0.009
Td	3	11.19	3.73	35.00	<.001
Ta.Td	6	1.48	0.24	2.32	0.069
Error	22	2.3	0.11		
Coefficient of variation	2.7				

vii). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of leaves per plant at 42 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	114.90			
Block	2	0.26	0.13	1.62	
Ta	2	10.74	5.37	64.53	<.001
Td	3	96.54	32.18	386.38	<.001
Ta.Td	6	5.51	0.91	11.03	<.001
Error	22	1.83	0.083		
Coefficient of variation	1.6				

viii). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of leaves per plant at 56 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	262.07			
Block	2	0.01	0.01	0.07	
Ta	2	3.67	1.83	22.98	<.001
Td	3	251.37	83.79	1047.72	<.001
Ta.Td	6	5.25	0.87	10.96	<.001
Error	22	1.75	0.07		
Coefficient of variation	1.1				

ix).Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of leaves per plant at 70 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	161.82			
Block	2	0.49	0.24	0.53	
Ta	2	11.78	5.89	12.55	<.001
Td	3	106.21	35.40	75.46	<.001
Ta.Td	6	33.01	5.50	11.73	<.001
Error	22	10.32	0.46		
Coefficient of variation	1.9				

x). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of leaves per plant at 84 Days after Planting

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	739.58			
Block	2	1.01	0.50	0.80	
Ta	2	511.04	255.52	403.90	<.001
Td	3	169.61	56.53	89.37	<.001
Ta.Td	6	43.99	7.33	11.59	<.001
Error	22	13.91	0.63		
Coefficient of variation	1.7				

xi). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on fruit length

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	99.68			
Block	2	0.08	0.04	1.59	
Ta	2	0.90	0.45	16.53	<.001
Td	3	96.52	32.17	1179.77	<.001
Ta.Td	6	1.56	0.26	9.56	<.001
Error	22	0.600	0.02		
Coefficient of variation	3.7				

xii). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on number of flowers

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	1278.05			
Block	2	0.01	0.004	0.08	
Ta	2	22.50	11.25	204.31	<.001
Td	3	1247.57	415.85	7551.55	<.001
Ta.Td	6	6.74	1.12	20.41	<.001
Error	22	1.21	0.06		
Coefficient of variation	1.5				

xiii).Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on fruits per plant

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	144.59			
Block	2	0.194	0.09	1.32	
Ta	2	10.18	5.09	69.12	<.001
Td	3	112.07	37.35	507.12	<.001
Ta.Td	6	20.51	3.41	46.41	<.001
Error	22	1.62	0.07		
Coefficient of variation	2.5				

iv). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on fruit diameter

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	30.004			
Block	2	0.10	0.01	0.18	
Ta	2	2.15	1.07	36.39	<.001
Td	3	25.87	8.62	290.67	<.001
Ta.Td	6	1.30	0.22	7.34	<.001
Error	22	0.65	0.03		
Coefficient of variation	5.3				

xv). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on fruit weight

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	351.12			
Block	2	0.24	0.12	3.48	
Ta	2	6.90	3.45	97.11	<.001
Td	3	339.39	113.13	3181.25	<.001
Ta.Td	6	3.79	0.63	17.77	<.001
Error	22	0.78	0.03		
Coefficient of variation	1.6				

xvi). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on yield per plant

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	105079.55			
Block	2	25.63	12.81	0.59	
Ta	2	1860.92	930.46	42.60	<.001
Td	3	99553.53	33184.51	1519.20	<.001
Ta.Td	6	3158.92	526.49	24.10	<.001
Error	22	480.55	21.84		
Coefficient of variation	3.4				

xvii).Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on Total soluble solids

Source	Df	Type III SS	MSE	F Value	P>F
Total	35	99.03			
Block	2	3.27	1.63	4.52	
Ta	2	2.23	1.11	3.08	0.066
Td	3	81.29	27.09	74.77	<.001
Ta.Td	6	4.26	0.71	1.96	0.115
Error	22	7.97	0.36		
Coefficient of variation	5.7				

xviii).Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on ascorbic acid content

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	854.32			
Block	2	3.27	1.63	0.86	
Ta	2	118.64	59.32	31.03	<.001
Td	3	598.70	199.56	104.41	<.001
Ta.Td	6	91.64	15.27	7.99	<.001
Error	22	42.05	1.91		
Coefficient of variation	2.8				

xix). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on diseases severity

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	21914.02			
Block	2	1.71	6.91	18.94	
Ta	2	19481.45	13270.09	36333.79	<.001
Td	3	1459.89	880.94	2412.05	<.001
Ta.Td	6	884.18	406.34	1112.59	<.001
Error	22	86.78	0.36		
Coefficient of variation	4.2				

xx). Effects of *Trichoderma asperellum* and *Tithonia diversifolia* on diseases incidence

Source	Df	Type III SS	MSE	F Value	P>F
		TRIAL			
Total	35	13767.74			
Block	2	50.06	25.03	1.30	
Ta	2	11844.43	5922.21	306.84	<.001
Td	3	968.26	322.75	16.72	<.001
Ta.Td	6	480.38	80.06	4.15	0.005
Error	22	424.62	19.30		
Coefficient of variation	8.9				

Appendix B: Field layout

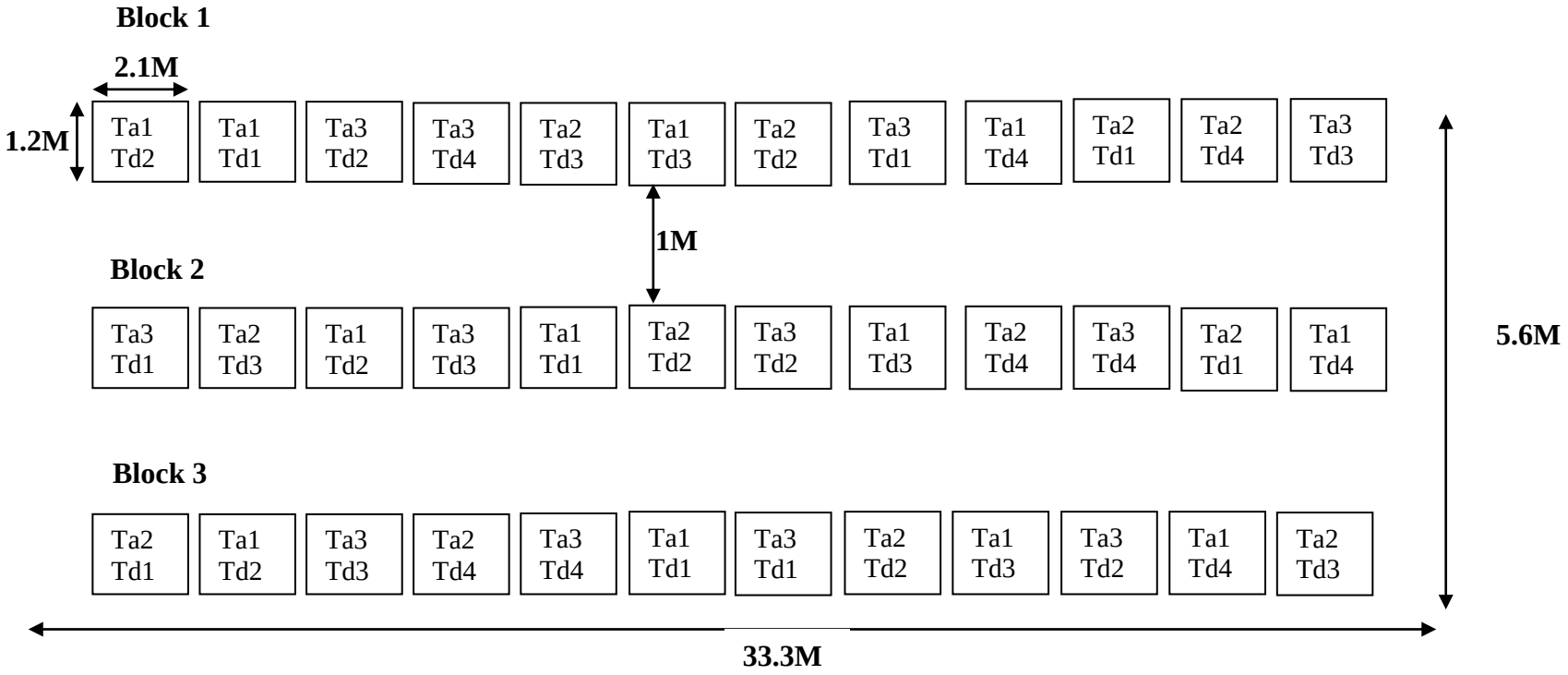

Figure 9: Experimental layout

KEY:

Ta - *Trichoderma asperellum*(**Ta1 -** 0mL, **Ta2-** 40mL and **Ta3 -** 80mL)

Td - *Tithonia diversifolia*(**Td1 -** 0mL, **Td2 -** 250mL, **Td3 -** 500mL and **Td4 -** 750mL)

Appendix C. Published Article

IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 13, Issue 8 Ser. I (August 2020), PP 50-55
www.iosrjournals.org

Effects of *Tithonia diversifolia* Extract and *Trichoderma asperellum* on Growth and Yield of Strawberry Fruit (*Fragaria× ananassavarDuch)*

Wanjukia, J.W.,[1]* Gesimba, R.M.[1]&Wolukau, J.N.[1]
1Department of Crops, Horticulture, and Soils, Faculty of Agriculture, Egerton University, P.O. Box 536-20115, Egerton, Kenya

**Corresponding Author: wambuijulia6@gmail.com*

Abstract
A study on the effect of Tithonia diversifolia extracts and Trichoderma asperellum on growth, yield, and quality of strawberry fruit (Fragaria× ananassavarDuch) was conducted in Horticulture, Research and Teaching field, Egerton University. The field experiment was laid out in a Randomized Complete Block design (RCBD), with 3 replications. There were two factors , Tithonia. diversifolia and Trichoderma asperellum. Tithonia diversifolia was applied at four concentrations (0mL/L, 250mL/L, 500mL/L, and 750mL/L) while Trichoderma. asperellum was applied at three levels (0mL, 40mL and 80mL per 20 liters of water). Data were subjected to Analysis of Variance (ANOVA) and significant treatment means separated using Turkey's Honestly Significant Difference Test at $P \leq 0.05$. Application of Tithonia diversifolia and Trichoderma asperellum increased plant height, number of leaves per plant, number of flowers per plant, number of fruits per plant, increased fruit length and diameter, and total yield per plant compared to the control.

Keywords: *strawberry, yield, Tithonia diversifolia,Trichoderma asperellum,. Brix,*

Appendix D. Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **940775**

Date of Issue: **05/October/2020**

RESEARCH LICENSE

This is to Certify that Ms.. JULIA WAMBUI WANJUKIA of Egerton University, has been licensed to conduct research in Nakuru on the topic: ``EFFECTS OF Tithonia diversifolia EXTRACT AND Trichoderma asperellum ON Botrytis cinerea INCIDENCE AND SEVERITY, GROWTH, YIELD AND QUALITY OF STRAWBERRY FRUIT (Fragariax ananassa var duch) for the period ending : 05/October/2021.

License No: **NACOSTI/P/20/6992**

940775

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is Guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014

CONDITIONS

1. The License is valid for the proposed research, location and specified period
2. The License any rights thereunder are non-transferable
3. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research
4. Excavation, filming and collection of specimens are subject to further necessary clearence from relevant Government Agencies
5. The License does not give authority to tranfer research materials
6. NACOSTI may monitor and evaluate the licensed research project
7. The Licensee shall submit one hard copy and upload a soft copy of their final report (thesis) within one year of completion of the research
8. NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice

National Commission for Science, Technology and Innovation
off Waiyaki Way, Upper Kabete,
P. O. Box 30623, 00100 Nairobi, KENYA
Land line: 020 4007000, 020 2241349, 020 3310571, 020 8001077
Mobile: 0713 788 787 / 0735 404 245
E-mail: dg@nacosti.go.ke / registry@nacosti.go.ke
Website: www.nacosti.go.ke