EFFICACY OF SELECTED FUNGICIDES AND BIO-CONTROL AGENTS
IN THE MANAGEMENT OF FUSARIUM WILT OF PASSION FRUIT

WASIKE MASINDE JACK
(B.Sc. (Hons))
REG: 156/11171/06

A Thesis submitted in partial fulfillment of the requirements for the award of
the Degree of Master of Science (Microbiology), Kenyatta University.

NOVEMBER
2013
DECLARATION

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

Signature: …………………………… Date: ……………………………

Wasike Masinde Jack
Department of Plant and Microbial Sciences
Kenyatta University, Kenya

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Signature: …………………………… Date: ……………………………

Prof. Ethel Monda
Department of Plant and Microbial Sciences
Kenyatta University

Signature: …………………………… Date: ……………………………

Dr. Ruth Amata
Department of Plant Pathology
National Agricultural Research Laboratories (NARL)
DEDICATION

This thesis is dedicated to my wife Martha and my children Keren and Caleb who have been a great source of motivation and inspiration.
ACKNOWLEDGEMENTS

I would like to record my great appreciation to my supervisors, Prof. Ethel Monda and Dr. Ruth Amata for their support and guidance during this project. They spent valuable time in reviewing my work from inception to completion. Their critical review has contributed to the success of this project. I deeply appreciate the material support received from Dr Ruth Amata without which the greater part of this work would not have been accomplished. I acknowledge moral support received from my colleagues Mr. James Githinji, and Mrs. Elizabeth Kago, who helped me to overcome numerous obstacles in my work. I express more gratitude to Mr. Ndirangu and Mr. Joseph Kinoti of the Kenya Agricultural Research Institute (KARI) for their technical and moral support in the laboratory. To all the other persons that I have not mentioned here, I will always cherish your great help and kindness. Thank you and may the almighty God dearly bless you all.
# TABLE OF CONTENTS

DECLARATION ............................................................................................................. ii

DEDICATION .............................................................................................................. iii

ACKNOWLEDGEMENTS ............................................................................................... iv

TABLE OF CONTENTS ................................................................................................. v

LIST OF TABLES ........................................................................................................ x

LIST OF FIGURES ...................................................................................................... xii

LIST OF PLATES ........................................................................................................ xiii

LIST OF APPENDICES ............................................................................................... xiv

ACRONYMS AND ABBREVIATIONS .......................................................................... xvi

ABSTRACT ................................................................................................................... xvii

CHAPTER ONE ........................................................................................................... 1

INTRODUCTION .......................................................................................................... 1

1.1 Background information ...................................................................................... 1

1.2 Problem statement ............................................................................................... 3

1.3 Justification .......................................................................................................... 4

1.4 Hypotheses ........................................................................................................... 4

1.5 Objectives ............................................................................................................ 4

1.5.1 General objective ............................................................................................ 4

1.5.2 Specific objectives ........................................................................................... 5

1.6 Significance of the study ..................................................................................... 5

CHAPTER TWO ........................................................................................................... 6

LITERATURE REVIEW ............................................................................................... 6
2.1 Origin, distribution and botany of passion fruit ........................................... 6
2.2 Importance of passion Fruit ........................................................................... 7
2.3 Cultivation of passion fruit ............................................................................ 8
2.4 Trellising and pruning .................................................................................. 9
2.5 Diseases of passion fruits ............................................................................ 10
   2.5.1 Brown spot .............................................................................................. 10
   2.5.2 Passion woodiness disease .................................................................... 11
      2.5.2.1 Symptoms of passion woodiness disease ...................................... 11
      2.5.2.2 Epidemiology of passion woodiness disease ................................. 11
   2.5.3 Blight ...................................................................................................... 12
   2.5.4 Fusarium wilt of passion fruit ................................................................ 12
      2.5.4.1 Symptoms of fusarium wilt ........................................................... 13
      2.5.4.2 Epidemiology of Fusarium oxysporum .......................................... 14
2.6 Management of fusarium wilt .................................................................... 15
   2.6.1 Cultural methods .................................................................................. 15
   2.6.2 Chemical control .................................................................................. 17
   2.6.3 Breeding ................................................................................................ 19
   2.6.4 Biological control ................................................................................ 20
   2.6.5 Integrated pest management (IPM) ...................................................... 22

CHAPTER THREE .............................................................................................. 24

MATERIALS AND METHODS .......................................................................... 24
3.1 Study site ...................................................................................................... 24
3.2 Collection of diseased plant samples .......................................................... 24
3.3 Isolation, identification and maintenance of *Fusarium oxysporum* f. sp. *passiflorae* .......................................................... 25

3.4 *In-vitro* tests: Evaluation of fungicides and bio-control agents against spore and mycelia growth of *Fusarium oxysporum* f. sp. *passiflorae* .......... 26

3.4.1 *In-vitro* test 1: Assessment of the effect of selected fungicides and bio-control agents on spore germination ................................................. 27

3.4.2 *In-vitro* test 2: Assessment of the effect of fungicides and bio-control agents on colony diameter ......................................................... 28

3.5 Preparation of fungal inoculum ........................................................................................................ 29

3.6 Inoculation techniques ...................................................................................................................... 29

3.7 Pathogenicity test and selection of highly virulent strains for use in efficacy trials .......................................................... 29

3.8 Disease assessment .......................................................................................................................... 30

3.8.1 Disease severity .......................................................................................................................... 30

3.8.2 Disease incidence ....................................................................................................................... 31

3.9 Crop performance assessment ..................................................................................................... 31

3.10 Greenhouse experiments .............................................................................................................. 32

3.10.1 Crop establishment .................................................................................................................. 32

3.10.2 Experimental design and treatment structure ......................................................................... 32

3.10.3 Evaluation of efficacy of fungicides and bio-control agents on fusarium wilt when applied as curatives ......................................................... 33

3.10.4 Evaluation of fungicides and bio control agents when applied as protectants ....................... 34
3.11 Data analysis .......................................................................................................................... 34

CHAPTER FOUR .......................................................................................................................... 35

RESULTS ..................................................................................................................................... 35

4.1 Effect of fungicides and bio-control agents on spore germination of

Fusarium oxysporum f. sp. passiflorae ............................................................................... 35

4.2 Effect of fungicides and bio-control agents on colony diameter of

Fusarium oxyoporum f. sp. passiflorae ................................................................................. 36

4.3 Effect of fungicides and bio-agents applied as curative and protectant on

passion fruit infected with Fusarium oxysporum f. sp. passiflorae ......................... 40

4.4 Effect of fungicides and bio-control agents applied as protectant and

curatives on fusarium wilt incidence (%) in passion fruit................................. 43

4.5 Effect of fungicides and bio-control agents on height of passion fruit

infected with Fusarium oxysporum f. sp. passiflorae .............................................. 46

4.6 Effect of fungicides and bio-control agents on the length (cm) of root

rot caused by Fusarium oxysporum f. sp. passiflorae on passion fruit............. 48

4.7 Effect of fungicides and bio-control agents on shoot dry weight (g) of

passion fruit infected with Fusarium oxysporum f. sp. passiflorae .............. 51

4.8 Effect of fungicides and bio-control agents on root dry weight (g) of

passion fruit infected with Fusarium oxysporum f. sp. passiflorae ........... 55

CHAPTER FIVE .......................................................................................................................... 58

DISCUSSION CONCLUSION AND RECOMMENATION .................................................. 58

5.1 Discussion .............................................................................................................................. 58

5.1.1 Effect of fungicides and bio-control agents on spore germination of
5.1.2 Effect of fungicides and bio-control agents on colony diameter of

Fusarium oxysporum f. sp. passiflorae ..........................................................59

5.1.3 Effect of fungicides and bio-control agents on fusarium wilt incidence, severity and root rot length of passion fruit ..............................60

5.1.4 Effect of fungicides and bio-control agents on height, shoot weight and root weight of passion fruit infected with fusarium wilt disease .... 63

5.2 Conclusions ........................................................................................................64

5.3 Recommendations ...............................................................................................65

REFERENCES ..........................................................................................................66

APPENDICES .............................................................................................................74
LIST OF TABLES

Table 2.1: Volumes of passion fruits exported over a six year period .................. 8

Table 2.2: Incidences of fusarium wilt of passion fruit in Kenya .................... 15

Table 2.3: Fungicides used to control fungal diseases in Kenya ..................... 18

Table 3.1: List of fungicides and bio-control agents as per manufacturer’s recommendation ................................................................. 26

Table 4.1: Spore germination (%) of *Fusarium oxysporum* f. sp. *passiflorae* treated with selected fungicides and bio-control agents after 12, 15 and 18 hours ........................................................................................................ 36

Table 4.2: Effect of fungicides and bio-control agents on percentage growth inhibition of colony diameter of *Fusarium oxysporum* f.sp. *passiflorae* from 3 to 7 day after inoculation ........................................... 39

Table 4.3: Effect of fungicides, bio-control and mode of application on severity of passion fruits infected with *Fusarium oxysporum* f. sp. *passiflorae*. (Trial 2) ................................................................. 42

Table 4.4: Incidence (%) of fusarium wilt on Passion fruits following application of fungicides and bio-control agents as protectants and curatives. (Trial 1) .................................................................................. 44

Table 4.5: Incidence (%) of Fusarium wilt disease on passion fruits following application of fungicides and bio-control agents as protectants and as well as curatives. (Trial 2) ........................................... 45

Table 4.6: Effect of fungicides and bio-control agents on the length (cm) of rot caused by *Fusarium oxysporum* f. sp. *passiflorae* on passion
fruits (Trial 1) ........................................................................................................49

Table 4.7: Effect of fungicides and bio-control agents on the length (cm) of
root rot caused by *Fusarium oxysporum* f. sp. *passiflorae* on
passion fruits (Trial 2) ..........................................................................................51

Table 4.8: Effect of fungicides and bio-control agents applied as protectants
and curatives on shoot dry weight of passion fruits in grams ..........54

Table 4.9: Effect of fungicides and bio-control agents applied as protectants
and curative on root dry weight of passion fruits (Trial 1).................56

Table 4.11: Effect of fungicides and bio-control applied as protectants and
curative on root dry weight of passion fruits (Trial 2) .......................57
LIST OF FIGURES

Figure 4.1: Effect of fungicides, bio-control and mode of application on severity of passion fruits infected with F. oxysporum f. sp. passiflorae (Trial 1) .................................................................41

Figure 4.2: Effect of fungicides, bio-control agents and mode of application on the height (cm) of passion fruits (Trial 1). ........................................47

Figure 4.3: Effect of fungicides, bio-control agents and mode of application on the height (cm) of passion fruits (Trial 2). ........................................48

Figure 4.4: Effect of fungicides, bio-control agents and mode of application on shoot dry weight (g) of passion fruits (Trial 1) .................................53
LIST OF PLATES

Plate 2.1: Dieback (left) and stem discolouration (right) caused by *Fusarium oxysporum*........................................................................................................... 14
LIST OF APPENDICES

Appendix 1: Spore germination (%) of *Fusarium oxysporum* f. sp. *passiflorae* treated with different fungicides and bio-control agents after 12, 15 and 18 hours..........................................................74

Appendix 2: Effect of fungicides and bio-control agents on mean mycelia inhibition% of *Fusarium oxysporum* f. sp. *passiflorae* from 3 to 7 day after inoculation..........................................................74

Appendix 3: Incidence (%) of fusarium wilt on passion fruits following application of fungicides and bio-control agents as curatives (Trial 1)..............................................................................75

Appendix 4: Incidence (%) of fusarium wilt on passion fruits following application of fungicides and bio-control agents as protectants (Trial 1)..............................................................................75

Appendix 5: Incidence (%) of fusarium wilt on passion fruits following application of fungicides and bio-control agents as protectants (Trial 2)..............................................................................76

Appendix 6: Incidence (%) of fusarium wilt on passion fruits following application of fungicides and bio-control agents as curative (Trial 2)..............................................................................76

Appendix 7: Effect of fungicides and bio-control agents applied as protectants on root dry weight of passion fruits (Trial 1) ..............................................77

Appendix 8: Effect of fungicides and bio-control agents applied as curative on root dry weight of passion fruits (Trial 1) ..............................................77
Appendix 9: Effect of fungicides and bio-control applied as protectants on root dry weight of passion fruits (Trial 2) ........................................78

Appendix 10: Effect of fungicides and bio-control applied as curative on root dry weight of passion fruits (Trial 2) ........................................78

Appendix 11: Effect of fungicides and bio-control agents applied as protectants on the length (cm) of rot caused by Fusarium oxysporum f. sp. passiflorae on passion fruits (Trial 1) ........................................79

Appendix 12: Effect of fungicides and bio-control agents applied as curative on the length (cm) of rot caused by Fusarium oxysporum f. sp. passiflorae on passion fruits (Trial 1) ........................................79

Appendix 13: Effect of fungicides and bio-control agents applied as protectants on shoot dry weight of passion fruits in grams (Trial 2) . 80

Appendix 14: Effect of fungicides and bio-control agents applied as curatives on shoot dry weight of passion fruits in grams (Trial 2) .... 80
## ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>CLA</td>
<td>Carnation Leaf Agar</td>
</tr>
<tr>
<td>FAO</td>
<td>Food Agricultural Organization</td>
</tr>
<tr>
<td>Fo</td>
<td><em>Fusarium oxysporum</em></td>
</tr>
<tr>
<td>HCDA</td>
<td>Horticultural Crop Development Authority</td>
</tr>
<tr>
<td>KARI</td>
<td>Kenya Agricultural Research Institute</td>
</tr>
<tr>
<td>MOA</td>
<td>Ministry of Agriculture</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PDA</td>
<td>Potatoe Dextrose Agar</td>
</tr>
<tr>
<td>SNA</td>
<td>Spezieller Nähstoffarmer Agar</td>
</tr>
<tr>
<td>TSP</td>
<td>Triple Superphosphate</td>
</tr>
</tbody>
</table>
Passion fruit (*Passiflora edulis* Sims) is an important fruit crop in Kenya for both local and export market. Production of the crop is constrained by many diseases. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *passiflorae* is one of the major disease of purple passion fruit. Yield losses due to wilt have been estimated up to 80%. Farmers lack appropriate management strategies for the disease and the use of bio-control agents to manage the disease has not been explored. The objective of the study was to evaluate the efficacy of selected fungicides and bio-agents in order to develop an integrated disease management package for management of fusarium wilt in purple passion fruit. *In-vitro* tests were conducted in the laboratory to evaluate the efficacy of selected fungicides Carbendazim, Thiophanate – methyl, Azoxystrobin, and Ridomil-Mz while bio-control agents were *T. harzianum* and *T. asperellum*. The control agents were evaluated in laboratory for their effect on inhibition of colony diameter and conidia germination of *F. oxysporium* f. sp. *passiflorae*. Laboratory experiments were arranged in completely randomized design with treatments replicated four times. Control plates were without treatments. Ridomil-Mz and Carbendazim significantly inhibited colony diameter growth at p=0.05 in laboratory. *T. asperellum* significantly inhibited colony diameter growth at p=0.05 in laboratory compared to *T. harzianum*. Laboratory experiments were followed by greenhouse experiments. The fungicides and bio-control agents were evaluated as per manufacturer’s recommendations. Purple passion fruit seedlings were raised in pots (18 cm diameter) in a greenhouse and the selected fungicides and bio-control agents were evaluated as curative as well as protectants for management of fusarium wilt. The experiments in greenhouse were arranged in split plot design with three replications per treatment. Control pots consisted of *F. oxysporium* f. sp. *passiflorae* inoculum and sterile distilled water. Plants (purple passion fruits) were assessed for disease severity and disease incidences whereas plant performance was tested on the basis of growth (height) and biomass production (shoot and root dry weights). The data obtained was subjected to ANOVA and means separated using LSD at P= 0.05. Results demonstrated that Carbendazim and Ridomil-Mz significantly reduced disease severity at p= 0.05 compared to other treatments when applied as protectants in greenhouse. *Trichoderma. harzianum* and *T. asperellum* significantly (p=0.05) led to low disease severity when applied as protectants compared to control. The two bio-agents also significantly (p=0.05) led to higher heights and higher biomass in greenhouse compared to controls. Application of Carbendazim and Ridomil- Mz as protectants as well as *T. harzianum* and *T. asperellum* can be integrated in the management of fusarium wilt of purple passion fruits in Kenya.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Passion fruit (*Passiflora edulis* Sims) is a native of southern Brazil, Paraguay and Northern Argentina (Njuguna *et al.*, 2005). Ecuador and Brazil are the world leading producers of passion fruits (Amata *et al.*, 2009). The fruit was introduced to Kenya in the 1920’s for commercial juice processing (HCDA, 2008, Farr *et al.*, 2013). In Kenya, area under production passion fruit is approximately 5,450 ha with an annual export production of about 1335 tons (Njuguna *et al.*, 2005). The purple passion fruit variety (*Passiflora edulis*), is the most important in the country and is grown for fruit juice and fresh export (HCDA, 2011; HCDA, 2012). Other varieties grown in Kenya include yellow passion fruit (*Passiflora edulis* var. *flavicarpa*), sweet passion fruit or sweet granadilla (*Passiflora ligularis*) and giant passion fruit or giant granadilla (*Passiflora quadrangularis*) (HCDA, 2008).

Currently in Kenya, passion fruit is grown in several districts on small holdings of East of Rift Valley (1200-1800 m above sea level) and in areas like Taita Taveta and Machakos, Middle zones of central highlands such as Thika, Embu, Meru and Nyeri. It is also grown West of Rift Valley (2000 m above sea level) in Kisii, Bungoma, Kakamega, Kitale, and Baringo (Njuguna *et al.*, 2005). The fruit is currently ranked third among fruit exports in Kenya and has great potential since demand for both fresh and processed fruit is on the rise (Amata and Otipa, 2008). Passion fruit is a good source of ascorbic acid (Vitamin C) and carotenoids...
(HCDA, 2008). It is also a popular constituent of cold drinks, cakes, ice cream and yoghurts (KARI, 2001). Increased production and consumption of the crop has been shown to improve health and nutritional status particularly in vulnerable groups such as children, the aged and HIV infected persons (Amata and Otipa, 2008).

Despite the economic importance of the crop in Kenya, the average yield is still relatively low at only 8 tons/ha compared to about 18.9 tons/ha in South Africa (Njuguna et al., 2005). One of the major constraints hindering sustainable crop production in East Africa is pests and diseases. In Kenya, the main diseases include, stem die back (*Fusarium* spp) anthracnose (*Colletotrichum passiflorae*), crown/collar rot (*Fusarium solani*), wilt (*F. oxysporum* f. sp. *passiflorae*), passion fruit woodiness virus disease complex (PWD), and root-knot nematodes (*Meloidogyne* spp) (Ssekyewa et al., 1999; Vieira and Carneiro, 2005; Njuguna et al., 2005). Insect pests such as aphids, thrips, sting bugs and white flies are also a major problem in passion fruit production (Njuguna et al., 2005). Yield losses of up to 80% have been attributed to a combination of these diseases and pests (KARI, 2001).

One of the most damaging of the diseases is fusarium wilt caused by *Fusarium oxysporum* f. sp. *Passiflorae* (Gardener, 2013) Currently the disease is being managed through grafting of the susceptible purple passion fruit as a scion onto the resistant yellow passion fruit used as a root stock (Ssekyewa et al., 1999; Vieira
and Carneiro, 2005). There is lack of information on the effectiveness of current pesticides in the market in the control of this disease. There is therefore a need to identify other management strategies of the disease such as selected fungicides and bio-control agents (Trichoderma harzianum and T. asperellum) in the management of fusarium wilt of passion fruit.

1.2 Problem statement
Passion fruit is a very important fruit in Kenya whose production is constrained by many diseases such as fusarium wilt which is caused by Fusarium oxysporum f. sp. passiflorae (Njuguna, 2005). Yield losses of up to 80% have been estimated (Amata et al., 2008). Currently the disease is being managed through grafting of the susceptible purple passion fruit as a scion onto the resistant yellow passion fruit used as a root stock (Amugune et al., 1993). The yellow root stock is unavailable to most of the farmers. Farmers who are using pesticides they are complaining about the pesticides being infective and yield loss due to fusarium wilt is high. There is lack of information on the effectiveness of current pesticides on the market for management of fusarium wilt disease in purple passion fruit and the use of environmentally friendly bio-agents (T. harzianum and T. asperellum) in the management of fusarium wilt of purple passion fruit has not been explored. There is hence an urgent need to take measures towards effective management of fusarium wilt.
1.3 Justification

Fusarium wilt is a devastating disease in purple passion fruit. Yield losses of up to 80% have been estimated (Amata et al., 2008). Farmers who are using pesticides are concerned about the chemicals being ineffective. There is limited information on the effectiveness of the current pesticide on the market and the use bio-agents (T. harzianum and T. asperellum) which are environmentally friendly (minimal environmental pollution) with minimal residues in human food, have not been explored. Therefore there is need to evaluate the efficacy of selected fungicide and bio-control and determine appropriate time of application in order to develop an integrated disease management package that has a high degree of food safety and minimal environmental impact, for management of fusarium wilt disease to minimize yield loss and increase purple passion fruit production.

1.4 Hypotheses

i. Selected fungicides, bio-control agents (T. harzianum and T. asperellum) are not effective in management of fusarium wilt of passion fruit.

ii. Application time does not affect efficacy of fungicides and bio-control agents in control of fusarium wilt of passion fruit.

1.5 Objectives

1.5.1 General objective

Efficacy of selected fungicides and bio-control in the management of fusarium wilt of passion fruits.
1.5.2 Specific objectives

i. To evaluate the efficacy of selected fungicides and bio-control agents in the management of fusarium wilt of passion fruits.

ii. To determine the appropriate time of application of selected fungicides and bio-control.

1.6 Significance of the study

The study will help to develop an integrated disease management package that has a high degree of food safety and minimal environmental impact, for management of fusarium wilt disease to minimize yield loss and increase purple passion fruit production.
CHAPTER TWO

LITERATURE REVIEW

2.1 Origin, distribution and botany of passion fruit

Passion fruit is a native of the tropics of southern Brazil. The fruit belongs to the family *passifloraceae* which contains 12 genera with about 500 species (Armstrong, 2013). The most important genus, *Passiflora*, contains 400 species of which about 50 to 60 species produce edible fruits which are palatable (Martin and Nakasora, 1994). Passion fruit is a perennial vigorous vine, which flowers and produces fruits within a year. The vine is shallow rooted, woody and climbs by tendrils. Fruits are oval or round with rough waxy rind. Fruit colours range from dark-purple with faint white specks to light yellow. The fruits contain a flavorful juice that is sub-acid to acid (HCDA, 2012; McKnight, 2013).

Two types of the *Passiflora edulis* have been highly cultivated. These types are the purple skinned *P. edulis* Sims and the yellow skinned *P. edulis* var *flavicarpa*. The latter type is considered higher yielding and more disease resistant than the former (FAO, 1972). Passion fruit is one of the most important tropical fruit species consumed worldwide as fresh fruit and juice or as an additive to ice cream and preserves. Although Brazil is reportedly the center of origin of passion fruit, it is now cultivated in all continents of the world. The main producers of the crop are Brazil, Colombia, Ecuador, Peru, Venezuela, Kenya, South Africa, Sri Lanka, Australia, New Zealand and Hawaii (Menzel *et al*., 1996). In Kenya, it is thought
to have been initially introduced by the European settlers in Kisii District (MOA, 2008).

2.2 Importance of passion fruit

Passion fruit is eaten fresh but the processors are the main commercial outlets. The fruit has several industrial uses including juice, pectin, oil extraction as well as cattle and poultry feed preparation (McKnight, 2013). It is grown commercially throughout the tropics and subtropics mainly by smallholder farmers (many of them women) who comprise 80% of growers (Vieira and Carneiro, 2005). In Kenya passion fruit has been grown commercially since the 1930’s and expanded in the 1960’s (Morton, 1987). Currently the fruit is mainly grown by smallholder farmers who have formed community based organizations (CBOs) contracted by export companies such as East African Growers and Kenya Horticultural Exporters for export (HCDA, 2012). The crop is currently ranked third among fruit exports but has the potential to lead because demand for both fresh fruit and processed juice is on the increase (MOA, 2003; Njuguna et al., 2005). As an export produce passion fruit has experienced an unstable trend over the years (Table 1) which is attributed to stringent market requirements. The most important variety in Kenya is the purple passion fruit *Passiflora edulis* Sims which is susceptible to fusarium wilt and is grafted on to yellow passion fruit root stock that is resistant to fusarium wilt (HCDA, 2012).
Table 2.1: Volumes of passion fruits exported over a six year period

<table>
<thead>
<tr>
<th>Year</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (Tons)</td>
<td>97,313</td>
<td>126,225</td>
<td>108,763</td>
<td>69,524</td>
<td>121,959</td>
<td>133,744</td>
</tr>
<tr>
<td>% Change</td>
<td>+22.9</td>
<td>-16</td>
<td>-56.4</td>
<td>+42.9</td>
<td>+8.8</td>
<td></td>
</tr>
</tbody>
</table>


Passion fruit is rich in vitamins A (700 mg / 100 g), riboflavin (0.13 mg / 100 g), niacin (1.5 mg / 100 g) and ascorbic acid (30 mg / 100 g), proteins (2.2 g / fruit), and minerals potassium (348 mg / 100 g), sodium (28 mg / 100 g), iron (1.6 mg/100 g), phosphorous (64 mg / 100 g) and calcium (13 mg / 100 g) (Morton, 1987). Increased production will lead to higher rural income, improved nutrition and health especially in vulnerable groups like children, the elderly and HIV/AIDS infected persons (MOA, 2004).

2.3 Cultivation of passion fruit

The optimum temperature range for growth and development of passion fruit is 25° C to 30° C. High temperature inhibits fruit set (HCDA, 2008). The crop requires a well distributed rainfall of 900 mm to 2,000 mm per annum (KEPHIS, 2008; MOA, 2012). Passion fruits grow on a wide range of soils which should be reasonably deep and fertile. It requires soil pH range of 6.0-6.5. In high rainfall areas, the soil should be well drained as the plant is intolerant to logging or flooding (Njuguna, 2005). Deep ploughing is encouraged to open up the soil for aeration and good water infiltration (KARI, 2007). Strict crop rotation should be
practiced to avoid build up of soil borne diseases. Spacing should be 2 cm between rows and 3 m within the rows (HCDA, 2008). Planting is by seed, stem cutting, or grafted propagules (HCDA, 2012). It is highly recommended to grow purple passion fruits grafted on yellow roots stocks to reduce *fusarium* wilt infestation (ICRISAT, 1998; Njuguna *et al.*, 2005). Planting holes of 45 cm x 45 cm x 45 cm should be dug well in advance (MOA, 2012; HCDA, 2012) with top soil and sub-soil separated. At planting, 175 g of Triple Super Phosphate (TSP) and a “debe” (about 20 kg) of farm yard manure are well mixed with top soil and returned to each hole (MOA, 2012). Top dressing should be done regularly with Calcium Ammonium Nitrate (CAN) at the rate of 300 g per plant per year distributed in two applications of 150g, each at the beginning of each rainy season (FAO, 2006; HCDA, 2008).

### 2.4 Trellising and pruning

Passion fruit is trained on a trellis constructed from wire and posts. The posts should be 270 cm high and 15 cm in diameter. The posts are placed in 60 cm deep holes spaced 6 m apart in the rows, midway between the plants (HCDA, 2007 and HCDA, 2008). All posts must be supported by an anchor (MOA, 2004). Old shoots, which are unproductive and all dead wood, must be removed as close to the main vine as possible to encourage the growth of new laterals (HCDA, 2007). Secondary shoots reaching the ground level have to be cut (HCDA, 2008). Heavy pruning should only be performed once per year, after the July to November crop.
2.5 Diseases of passion fruits

Diseases are highly rated among the principal constraints to sustainable crop production in East Africa (Sutherland and Kibata, 1993). Global losses attributed to plant diseases are approximated at 24.8 million dollars annually (Olanya et al., 2001). Whereas the crop is attacked by one or two economically important pests or diseases in temperate climates, multiple pest infestations and/or disease infection are the norm within the tropics and subtropics thus compounding their management (HCDA, 2005). In Kenya, yield losses of up to 80% have been attributed to a combination of diseases and pests including root rots (Fusarium spp.), nematodes (Meloidogyne spp.), passion fruit woodiness disease (PWD) complex, aphids, thrips and stink bugs (KARI, 2001).

2.5.1 Brown spot

Brown spot is caused by Alternaria passiflorae. It is a soil borne fungal first seen on the lower leaves (Mbaka et al., 2006) It attacks leaves and fruits causing angular, irregular spots with brown margins, with a lighter, often cracked centre (HCDA, 2007). On fruit, pin like sunken spots emerge, later enlarging into sunken circular spots with brown centers. The rind round the diseased part wrinkles and shriveled fruit drops conditions (HCDA, 2008). It occurs mainly during alternating periods of wet, dry and warm weather The Alternaria fungus infects, sporulates on dead plant tissue and re-infects the fruit, stem and leaves (Njuguna et al., 2005). It is spread by wind and rain splash. The disease can cause
severe defoliation and stem dieback, and can infect fruit at any time (Njuguna et al., 2005).

2.5.2 Passion woodiness disease

The disease is caused by woodiness pot virus (Nakasone and Paull, 1998). The disease leads to distortion of leaves and woodiness of fruits. Plants are stunted, yield reduces and vine dies (HCDA, 2012). According to Mali and Khalikar (1977), PWD and brown spot diseases are the most devastating disease of passion fruit.

2.5.2.1 Symptoms of passion woodiness disease

Temperature influences the development of fruit and leaf symptoms (Gardener, 2013). Symptoms are more common on fruit that set during autumn and winter to early summer and on leaves they are less pronounced in temperate conditions. Leaf mosaic, mottle and ring spot symptoms are often associated with fruit woodiness. Mosaic symptoms appear as dark green, raised blisters on a yellow-green background (Farr et al., 2013). Leaves are hardened and frequently distorted and puckered by the differential growth rates of the infected tissue. Vein clearing may also occur.

2.5.2.2 Epidemiology of passion woodiness disease

The disease is transmitted by aphids and by pruning tools; A regular spray program should be in place to control the sap sucking insects. Chemical control of
vectors (Aphids) easier as they are ‘visitors’ and do not colonize passion fruit plants.

2.5.3 Blight
Passion fruit blight is caused by *Phytophthora nicotianae*. The symptoms are dark water soaked lesions on leaves and diseased fruits fall prematurely (FAO, 2004).

2.5.4 Fusarium wilt of passion fruit
Fusarium wilt is a serious disease caused by the fungus *Fusarium oxysporum* f. sp. *passiflorae* (Gardener, 2013). Other fungi in this genus have been reported to cause other diseases in different crops, these include *F. solani* that causes crown rot (Njuguna *et al*., 2005), *Fusarium subglutinans* f. sp. *pini* the cause of pitch canker (Britz *et al*., 1999), *F. semitectum* that causes post-harvest losses in bananas and other fruits (Burgess *et al*., 1994; Nirenberg *et al*., 1998). The genus *Fusarium* occurs in most climatic regions of the world (Backhouse *et al*., 2001) and is common in soil, aerial plant parts, plant debris and other organic substrates where they survive mainly as chlamydospores or resistant hyphae (Burgess, 1981; Burgess and Summerell, 1992; Burgess *et al*., 1994; Summerell *et al*., 2003). Some species can colonize plants endophytically without causing symptoms of disease, but can cause diseases in the plants if the plants are subjected to moisture stress or other stresses (Trimboli and Burgess, 1983).
Other species are pathogenic and saprophytic found in association with plants in agricultural and natural ecosystems (Burgess, 1981; Summerell et al., 2003; Leslie et al., 2004). Although the genus is distributed widely, studies on *Fusarium* species in soils in various climatic regions in Australia, for example have shown that climate has a significant influence on the spectrum and abundance of *Fusarium* species associated with soils and plants in specific geographic areas (Burgess, 1981; Backhouse et al., 2001; Backhouse and Burgess, 2002). Most research on fusarium has been focused on species that are pathogenic to crop plants because of their economic importance (Smith et al., 1988).

### 2.5.4.1 Symptoms of fusarium wilt

The symptoms of fusarium wilt first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves (Gardener, 2013) At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988). Browning of the (vascular tissue) is strong evidence of fusarium wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Jones et al., 1982; Smith et al., 1988).
2.5.4.2 Epidemiology of *Fusarium oxysporum*

*F. oxysporum* is primarily spread over short distances by irrigation water and contaminated farm equipment (Armstrong, 2013). The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 1988). It is also possible that the spores are spread by wind (Farr et al., 2013). In Kenya, most passion fruit growing areas have been infested by *F. oxysporum* f. sp. *passiflorae* (Amata and Otipa 2008). According to Amata et al, (2008) the highest incidences of fusarium wilt of passion fruit were observed in Nakuru and Bungoma counties while none was found in Kisii County (Table 2). Complete absence of fusarium wilt in disease in Kisii district of Nyanza province may imply that the local ungrafted purple variety predominantly grown in Nyanza is tolerant to the wilt disease or that the farmers

---

**Plate 2.1:** Dieback (left) and stem discolouration (right) caused by *Fusarium oxysporum*.  
Source: Amata and Otipa (2008).
control the disease by planting clean seedlings and ensuring high levels of field sanitation (Amata et al., 2008).

### Table 2.2: Incidences of fusarium wilt of passion fruit in Kenya

<table>
<thead>
<tr>
<th>District (County)</th>
<th>Percentage incidences (upper limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nakuru</td>
<td>33</td>
</tr>
<tr>
<td>Bungoma</td>
<td>20</td>
</tr>
<tr>
<td>Embu</td>
<td>14</td>
</tr>
<tr>
<td>Thika</td>
<td>13</td>
</tr>
<tr>
<td>Meru</td>
<td>13</td>
</tr>
<tr>
<td>Molo</td>
<td>13</td>
</tr>
<tr>
<td>Kisii</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: Amata et al., 2008.

### 2.6 Management of fusarium wilt

#### 2.6.1 Cultural methods

Currently, yellow passion fruit seedlings are used as rootstocks because they offer short term resistance to fusarium wilt. Although the family *passifloraceae* has more than 500 species, limited research has been done to find an alternative rootstock for purple passion fruit production. Resistant rootstocks are needed because fusarium wilt control using methyl bromide fumigation is now banned worldwide because of its greenhouse gas effect. Also, fumigation is not economically feasible for small-scale producers especially in Kenya. Grafting aimed at taking advantage of the fusarium resistant yellow passion fruit is an established cultural practice in passion fruit production in Kenya. Several grafting
techniques have been used in other crops. These techniques include whip, splice and cleft grafting. The cleft grafting method is the oldest and the most widely used method of grafting (Hartman et al., 1994). Cleft grafting is popular with passion fruit propagators because it gives excellent results (Gardener, 2013). Cleft grafting is relatively inexpensive and simple to make (Farr, 2013). Compatibility refers to the ability of the scion and the rootstock to join and grow as one plant (Hartman et al., 1994). Usually, incompatibility results when the scion and root stock are distantly related genetically.

The family *passifloraceae* has over five hundred wild species and most of them produce few seeds (Ulmer et al., 2004). The simplest method of propagating *passiflora* species asexually needs to be developed before screening for graft compatibility with the edible varieties (Njeguna et al., 2005) Propagation by cuttings is the simplest and most widely used method of asexual propagation (Njuguna et al., 2005). Most species in the genus *Passiflora* grow rapidly and offer almost unlimited material for cuttings (Ulmer et al., 2004). Best results are from vines that are more than one year old, especially in the case of hybrids (Hartman et al., 1994). Cuttings need a temperature of 25°C to root but members of the subspecies *Teconia* need temperatures ranging from 15 to 20°C. (Ulmer et al., 2004). Propagation followed by screening *passiflora* species for fusarium wilt resistance and compatibility with the purple passion fruit, may result in identifying other species that can be used as a rootstock for the purple passion fruit in Kenya(Ulmer et al., 2004). Soil nutrition involves the use of nitrogenous fertilizers
to enhance plant growth (Njuguna et al., 2005). Use of soil amendments such as liming is a useful cultural practice that has been used over time to control pests and diseases (Njuguna, 2005).

2.6.2 Chemical control

Chemical control of fusarium wilt in passion fruit entails the use of fungicides which may be protective or systemic (Munene, 2003). It is recommended that protective fungicides be alternated with systemic fungicide to reduce resistance by the pathogen (Njuguna et al., 2005). Soil borne fungal pathogens like *Fusarium* require that fungicides are applied to the soil as dusts, liquid drenches or granules (Wangichunge, 1998). Fumigants are also applied usually before planting (Wangichunge, 1998). Problems associated with fungicide, for example toxicity contamination of food and water (FAO, 1988), there has been a move by some countries (Europe and N. America) to restrict or eliminate their use entirely (Munene, 2003). Thus the European commission in its white paper, has declared 50 % reduction in pesticide use as a policy objective for the European union The Commonly used fungicides in the control of fungal diseases including fusarium wilt in Kenya are as shown in Table 3.
### Table 2.3: Fungicides used to control fungal diseases in Kenya

<table>
<thead>
<tr>
<th>Fungicide (active ingredient)</th>
<th>Family</th>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-methyl</td>
<td>Thiophanate</td>
<td>Inhibit mitosis and cell division</td>
<td>(Agrios, 2005)</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>Acylanines</td>
<td>Disrupts nucleic acid synthesis</td>
<td>(Kohle et al., 2007)</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>Dithiocarbamate</td>
<td>Inactivates enzymes</td>
<td>(Wangichunge, 1998)</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>β-Methoxyacrylates</td>
<td>Inhibition of electron transport</td>
<td>(Agrios, 1988)</td>
</tr>
</tbody>
</table>

**Source:** Agrochemicals, 2005.

One of the documented studies of these fungicides against fusarium wilt is Azoxystrobin. The study showed the fungicide exhibited a high efficacy on fusarium wilt of three ornamental crops namely carnation, cyclamen and paris daisy. Azoxystrobin was shown to be similar or better than benomyl applied at higher dosages in all trials (Gullino et al., 2001). Literature on use of Carbendazim on controlling fusarium wilt is available in Equador and India only, yet these two show significant differences. (Morton, 1987) In Ecuador, the form available is soluble concentrate with a concentration of 50% (Munene, 2003). On the other hand, Carbendazim available in India is a wettable powder whose concentration is 50% (Munene, 2003). Equador gives a post-harvest interval of 3 days while India does not (Morton, 1997). Fungicide testing is important before application on passion fruits (Njuguna et al., 2005).
In Kenya farmers growing passion fruits for export use fungicides recommended by Global gap standards (Amata et al., 2008). It is important to evaluate new fungicides on the market for use by passion fruit growers (Amata et al., 2008). Passion fruit growers face difficulties due to little or no knowledge of pest and disease scouting as well as fungicide application methods (Njuguna et al., 2005).

2.6.3 Breeding

Fusarium wilt disease has made it necessary to graft susceptible cultivars onto fusarium resistant rootstocks (Ssekyewa et al., 1999; Vieira and Carneiro, 2005). The yellow passion fruit is used as the root stock due to its resistance to fusarium wilt disease, while the purple is used as the scion (Njuguna et al., 2005). Efforts are being put locally to find alternative passiflora species that are both compatible with the purple passion fruit and tolerant to fusarium wilt (Munene, 2003; Hawksworth, 1983). The absence of fusarium wilt in Kisii County may imply that the local ungrafted purple variety predominantly grown in the region is resistant (Amata et al., 2009). It may be necessary to establish this finding.

Breeding for new resistant root stocks is being done in Australia (Ulmer et al., 2004). Important results of breeding for resistance are the hybrid purple passion fruit which show considerable fusarium wilt resistance. However, there are reports of new virulent Fusarium races that appear within a few years after commercialization of resistant cultivars (Alabouvette et al., 2012).
2.6.4 Biological control

Biological control is the use of specific microorganisms to control plant pathogens and pests, it is an environmental friendly method meant to overcome problems caused by use of chemical pesticides (Chet and Brotman 2006) and usually isolated from suppressive soils (Barbosa et al., 2001). One of the most widely used beneficial microorganisms is *Trichoderma* (Biljana and Yugoslav, 2011).

The genus *Trichoderma* is made of filamentous fungi widely distributed in soils, plant material, decaying vegetation and wood. This genus has seven species namely *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii*, *T. viride*, *T.asperelum* and *T. citrinoviride* (Kuhls et al., 1999; Faheem et al., 2010). Species of *Trichoderma* are well known bio-control agents of soil-borne plant pathogenic fungi (Biratu et al., 1990; Onsando, 1991).

The increased growth response of several plants, following application of *Trichoderma* spp. to soil under both greenhouse and field conditions, has also been well documented (Baker, 1989; Kleifeld and Chet, 1992; Bailey and Lumsden, 1998; Pandya et al., 2011). The antifungal abilities of *Trichoderma* have been subjected to extensive efforts to use it as an antagonist to *Armillaria* since the 1914. However significant findings were made by Dumas and Boyonoski in 1992 with regards to the mode of action of *Trichoderma* species. Some species of *Trichoderma* have been shown to parasitize other fungi, out-compete them for nutrients and produce antibiotics (Harman, 1996; Klein and Everleigh, 2009).
These qualities facilitate the species capacity to colonize particular habitats in which they may hamper development of various fungal species including plant pathogenic ones.

*Trichoderma* grow tropically towards hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes, a process called mycoparasitism that limits growth and activity of plant pathogenic fungi (Yedidia *et al*., 1999).

Certain strains of this fungus colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant that leads to induced systemic resistance in the entire plant as demonstrated using cucumber (Chet *et al*., 2006). The capability of *T. harzianum* to promote increased growth response in cucumber was verified both in greenhouse experiments and in the hydroponic system (Ramot *et al*., 2004). A 30 percent increase in seedling emergence was observed and these cucumber plants exhibited a 95% rise in root area (Chet *et al*., 2009). Similarly, an increase in P and Fe concentration was observed in *Trichoderma* inoculated plants. The fungus *T. asperellum* has been shown to have the ability to store a high amount of lytic enzymes in an active form and secrete into the medium later. Examples of how some *Trichoderma* species have effectively controlled plant pathogens include suppressing the growth of mycelia of *Alternaria alternata* by more than 55% in post-harvest management of berry (*Ziziphus mauritiana* Lamk) fruit rot (Chet *et al*., 2009).
Similarly, introduction of *Trichoderma* spp into the soil substrate led to an increase in dry biomass production of cucumber plants (Wojtkowiak-Gębarowska and Pietr, 2006). This same experiment demonstrated that plant growth stimulation was associated with improved colonization of the root systems by *Trichoderma* isolates with the best colonizers occupying the root systems in the range of 227 to 1159 CFU per 1 g of soil and stimulated growth by 41–106%.

It was also observed that *Sclerotinia sclerotiorum* and *Fusarium culmorum* were reduced on roots of the cucumber plants. Ozbay and Newman (2004) demonstrated that *T. harzianum* improved tomato seedling growth in soil-less conditions under a greenhouse. The use of antagonistic microorganisms to overcome *F. oxysporum* f. sp. *passiflorae* in passion fruit in Kenya is still on trial. Since these microorganisms suppress the development of the pathogenic *Fusarium* types just as has been demonstrated for other crops, they represent an alternative method of suppressing *Fusarium* populations in soil and therefore managing fusarium wilt in passion fruit (Alabouvette *et al.*, 2012). However, this method has been successful only in greenhouse environments with crops grown in soil-less substrates in containers (Dumas and Boyonosonki, 1992).

### 2.6.5 Integrated pest management (IPM)

Combining various pest and disease control strategies has been promoted over the past few years in what is commonly referred to as Integrated pest management (IPM) (Njuguna *et al.*, 2005; Birch, 2001). Several studies have shown that a
combined application of chemical and biological agents for controlling plant pathogens appears to be a solution which can reduce pesticide input into environment as well as provide a better phytopathogen control (Cook, 1988; Elad and Shtienberg, 1994; O’Neill et al., 1996).

Greenhouse studies conducted to determine if disease suppression of fusarium wilt of cyclamen could be enhanced by combining fungicides and biological showed that when bio-agents were used as the first preventative treatment and inoculated a week later, no significant control was observed even after the crop was returned to fungicide program. (Cook, 1988; Elad and Shtienberg, 1994; O’Neill et al., 1996). However, when bio-agents were applied after or with fungicides, significant reduction of the disease was observed (Elmer and McGovern, 2004). The study concluded that the efficacy of integrating bio-agents with fungicides has potential, but more studies were necessary to identify specific combinations between bio-agents and fungicides that improve plant health. It is beneficial to use this approach because it will reduce the number of chemical sprays, reduce development of pest resistance to fungicides, improve plant growth and quality and lessen environmental hazards (Elmer and McGovern, 2004). Ongoing research in Kenya is geared towards finding an integrated disease management strategy that can be used to manage fusarium wilt of passion fruit under field conditions. It is postulated that this in combination with properly tested and selected fungicides will result in making passion fruit production economically viable.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site
The study sites were Thika, Embu and Kirinyaga. Thika is located in Kiambu County, it lies about 1200 m above sea level and occupies about 171 sq Km and has savannah type of climate. Embu County lies between 300 m and 5199 m above sea level and has equatorial type of climate. It receives annual rainfall of 1800 mm. Kirinyaga County, lies about 1300m above sea level. Annual rainfall in Kirinyaga County is about 1500 mm. The most dominant soils are stony loam soils.

3.2 Collection of diseased plant samples
Diseased passion fruit plants having fusarium wilt symptoms which include vein clearing on the outer young leaflet, followed by epinasty of petioles. On older plants, stunted growth, yellowing of the lower leaves, browning of vascular system, root rot, dropping of older leaves, leaf and stem wilting from the girdling of the lower part and death, reduced yield and at an advanced stages permanent wilt of the leaves which die as they cling to the upright woody stem (Agrios, 1988). The infected plants were collected from passion fruit growing areas in Thika (3 farms), Embu (3 farms) and Kirinyaga (3 farms). These areas were selected because of having large farms under passion fruit production and also due to high fusarium wilt incidences. The samples were packed in brown paper bags and transported to National Agricultural Research Laboratory (NARL) in Westland’s Nairobi, where they were stored at 4°C for 14 days.
3.3 Isolation, identification and maintenance of *Fusarium oxysporum* f. sp. *passiflorae*

Infected passion fruit stems were cut into one centimeter pieces and surface sterilized with 1% sodium hypochlorite for one minute and rinsed in three changes of sterile distilled water, then dried between sterile filter papers. Sterilized pieces were cultured on Peptone PCNB Agar (PPA) for 7 days; sub cultured on carnation leaf agar (CLA), and single spored onto Potato Dextrose Agar (PDA) and incubated at 25°C for four days.

Pure cultures were further obtained by sub culturing and single sporing. Single pure colonies were picked and used for fungal identification. Conidia morphology was observed on CLA, colony pigmentation was observed on PDA. The identified cultures were stored in 15% glycerol at -20°C. Fungi were identified according to Nelson *et al*, (1983), and Leslie and Summerrel (2006).
Table 3.1: List of fungicides and bio-control agents as per manufacturer’s recommendation

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Common name</th>
<th>Mode of action</th>
<th>Rate of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate -methyl (a)</td>
<td>(Topsin-M)</td>
<td>Disrupts cell division</td>
<td>20 ml in 20 ltrs of water for 1 ha</td>
</tr>
<tr>
<td>Carbendazim (a)</td>
<td>(Bavistin 50 DF)</td>
<td>Interferes with nuclear division</td>
<td>20 ml in 20 ltrs of water for 1 ha</td>
</tr>
<tr>
<td>Azoxystrobin (a)</td>
<td>Ortiva</td>
<td>Inhibits electron transport</td>
<td>20 ml in 20 ltrs of water for 1 ha</td>
</tr>
<tr>
<td>Metalaxyyl+mancozeb (a)</td>
<td>(Ridomil-Mz)</td>
<td>Inhibits RNA polymerase/inactivates enzymes</td>
<td>50 g in 20 ltrs of water for 1 ha</td>
</tr>
<tr>
<td>\textit{Trichoderma harzianum} (b)</td>
<td>(Bioderma)</td>
<td>Myco-parasitism, competition</td>
<td>10 g in 1 litre per 100 m$^2$</td>
</tr>
<tr>
<td>\textit{Trichoderma asperellum} (b)</td>
<td>(Bioderma)</td>
<td>Myco-parasitism, antagonistic</td>
<td>125 g in 1 litre per 100 m$^2$</td>
</tr>
</tbody>
</table>

Source: (a) Agrochemicals, 2005; (b) Juanco, 1997.

3.4 \textit{In-vitro} tests: Evaluation of fungicides and bio-control agents against spore and mycelia growth of \textit{Fusarium oxysporum} f. sp. \textit{passiflorae}

Two \textit{In-vitro} tests were conducted once at National Agricultural Research Laboratories to determine efficacy of four different selected fungicides (Carbendazim, Ridomil-Mz, Azoxystrobin and Thiophanate - methyl) and two bio-control agents (\textit{T. harzianum} and \textit{T. asperellum}) against \textit{F. oxysporum} f. sp. \textit{passiflorae}. Effects of these fungicides and bio-control agents on mycelia growth and spore germination were assessed, with a view of obtaining data that would assist farmers on most appropriate fungicides and bio-control that can be used
effectively for the management of fusarium wilt. The fungicides and bio-control agents tested are shown in (Table 3.1). Sterile distilled water was used for control experiment.

3.4.1 *In-vitro* test 1: Assessment of the effect of selected fungicides and bio-control agents on spore germination

*Fusarium* spore suspension of $10^6$ spores per milliliter was used. A drop (0.04 ml) of the spore suspension containing $4 \times 10^4$ spores was put on cavity slides consisting of approximately 1 drop of each of the four selected fungicides and two bio-control agents diluted according to the manufacturer’s recommendation (Shitabule, 2005). The fungicides were Carbendazim, Thiophanate-methyl, Ridomil-Mz, and Azoxystrobin, and the bio-control agents were *Trichoderma harzianum* and *T. asperellum*. All the cavity slides were placed individually in sterile Petri-dishes lined with moist sterile filter paper and incubated at room temperature (22-25° C).

Control slides contained *Fusarium* spores in sterile distilled water. There were four replicates per treatment arranged in a completely block design. Germination of conidia (visible germ tube) was counted under light microscope after 12, 15 and 18 hours of incubation. Percentage germination was determined according to (Fitzel, 1981). The germinated spores were expressed as a percentage of the total number of spores counted per ml as shown in the equation below:-

\[
\% \text{ spore germination} = \frac{\text{Total number of germinating spores in suspension}}{\text{Total number of examined spores in suspension}} \times 100
\]
3.4.2 *In-vitro* test 2: Assessment of the effect of fungicides and bio-control agents on colony diameter.

This test was conducted using PDA treated separately with Carbenadazim (0.5ml per 500 ml), Ridomil-Mz (0.3 ml per 500ml), Thiophanate-methyl (0.5 per 500 ml) Azoxystrobin (0.5ml per 500 ml) and Bio-control agents *T. harzianum* (0.5 ml per 500 ml) and *T. asperellum* (0.5 ml per 500 ml). Fungicides were diluted as per manufacturer’s recommendations (Table 4) Fungicides and bio-control agents were incorporated in PDA by preparing appropriate fungicides and bio-control dilutions and dispensing in appropriate volume of PDA contained in a conical flask at 45° C and later dispensing approximately 20 ml into Petri dishes as described by Owino et al. (1993). One mycelia plug (5 mm) from the periphery of 10 old fungal cultures was transferred aseptically to the center of amended PDA plate (one plug per plate). The dishes were incubated for 10 days at room temperature (22-25° C). Petri dishes with neither fungicides nor bio-control agents acted as control. Each treatment was replicated four times in a complete block design. Diameters of fungal colonies were measured using Vanier caliper to the nearest millimeter (mm) on 3, 4, 5, 6, and 7 days after inoculation. The inhibitory activity of fungicides and bio-control agents was determined by calculating the inhibition percentage (1%), (Nishijima and Smalley, 1979).

\[
\text{Inhibition } \% = \frac{C - T}{C - M} \times 100
\]

Where,

\(C= \text{ diameter (mm) of fungal colony on control plate}\)

\(T = \text{ diameter (mm) of fungal colony on fungicide / bio-control treated plates}\)

\(M = \text{ initial diameter (mm) of fungal colony}\).
3.5 Preparation of fungal inoculum

*Fusarium* spores cultured on CLA were used as inoculum. Conidia suspension were obtained by flooding ten day old culture with sterile distilled water, scraping the surface with a wire loop and filtering the suspension on two layers muslin clothes and centrifuged at 1200 rpm for 15 minutes to remove fungal mycelium. Conidia suspensions were adjusted to $10^6$ spores per milliliter using a haemocytometer (Owino *et al.*, 1996).

3.6 Inoculation techniques

The soil infestation method described by Prasad and Weigler (1976) Schuster and Coyne (1974) was used to inoculate soil in green house. For inoculation, 5 ml of distilled water containing $1.0 \times 10^6$ spore / ml of *F. o f. sp passiflora* was pipetted into planting pots (18 cm in diameter) containing sterilized soil. For curative assessment the inoculum was applied 14 days before transplanting to allow inoculums build up while for protectant assessment the inoculum was applied immediately after transplanting (Wangichunge, 1998).

3.7 Pathogenicity test and selection of highly virulent strains for use in efficacy trials.

*Fusarium oxysporum* f. sp. *passiflora* isolated from diseased purple passion fruit stems as described in section 3.3 was subjected to pathogenicity test to identify more virulent isolates for use in efficacy trials. Purple passion fruit seedlings were raised from certified seeds in pots in greenhouse. Fertilizer (D.A.P, 18-46-0) was used at planting time at the rate of 2 g per pot. The green house was kept free from
diseases and pests by sterilizing the greenhouse with 1% hypochlorite and use of Dimethoate respectively.

The fungal inoculum prepared as described in section 3.5 was used. The inoculation technique described in section 3.6 was used to apply the inoculum to sterile soil contained in planting pots. The inoculated soil was left for 14 days to allow the inoculum build up before transplanting seedlings. One disease free healthy seedling (5 weeks old) selected from stock seedlings was transplanted to each planting pot, in total 10 selected plants were used for the pathogenicity test. The plant symptoms as described in section 3.8.1 were observed 14 days after transplanting. Disease development was monitored in terms of disease severity (Sutherland et al., 1996).

3.8 Disease assessment

3.8.1 Disease severity

Disease severity was assessed starting from the second week after transplanting by examining symptoms which include drooping and pale colored leaves, leaves collapse to prostate position, shrunken stems both and above below ground and chlorosis of the whole plant. Data was collected on a weekly basis for eight weeks using a scale of 0-3 as described by Sutherland et al. (1996), where;

0 = No wilt infection (no yellow spots)
1 = <10% of plant parts showing symptoms (one or two leaves are yellow).
2 = 10-30% of plant parts showing symptoms (two to three leaves yellow)
3 = >30% of plant parts showing symptoms (extensive chlorosis)
The length of discolored root was measured in centimeters (cm) using a ruler and the length recorded to the nearest cm. The observed values were averaged to obtain a mean length of discolored root tissue (MLDRT). There were two main plots. Six treatments and one control were used in experiment. Each treatment was replicated three times, each replica was represented by three plants. There were 54 plants for curative application and 9 plants were used to represent control giving a total of 63 plants. For protectant application there were also 54 plants, 9 plants represented control, giving a total of 63 plants. The total number of plants used in greenhouse experiment was 126 purple passion fruit plants.

### 3.8.2 Disease incidence

Disease incidence was assessed by examining the number of plants infected in a given treatment and expressing this as a percentage of total number of plants.

### 3.9 Crop performance assessment

Effects of treatments on passion fruits growth were assessed by measuring shoot height (cm), dry weight of shoot and root. Shoot height was measured from the soil surface to upper most leaf apex (Wangichunge, 1998). Height was measured every week starting from the 6th week of age up to 14th week of age. Dry weight of shoots and roots were obtained by drying the materials at 80°C for 72 hours before weighing. The weights were taken after the 14th week of age (more than three quarters of plants were showing fusarium symptoms). The roots were washed to remove the soil before drying them.
3.10 Greenhouse experiments

Greenhouse experiments were conducted at National Agricultural Research laboratories (NARL) Nairobi Kenya between January to December, 2010. The greenhouse experiments were repeated once. The purple passion fruit *Passiflora edulis* (Sims) was chosen because it’s widely grown in Kenya and is highly susceptible to fusarium wilt.

### 3.10.1 Crop establishment

Purple passion fruit seedlings (stock seedlings) were raised from certified seeds in plastic pots in greenhouse at National Agricultural Research laboratories (NARL). The sterilized soil, sand, ballast and manure mixed in the ratio of 2:1:1:1 (weight: weight) was obtained from the Kenya Agricultural Research Institute (KARI) at Muguga. Fertilizer (D.A.P, 18-46-0) was used at planting time at the rate of 2 g per pot. The greenhouse was kept free from diseases by sterilizing it using 1% hypochlorite and use of Dimethoate to control aphids and thrips. Other agronomic practices were carried out according to recommended agricultural practices.

### 3.10.2 Experimental design and treatment structure

The greenhouse experiment was laid as a split-plot design with three replications. The main plots were treatments with selected fungicides and bio-control agents at the recommended rates whereas the subplots were time of treatment application that is application of fungicides and bio-control agents prior to infection (protectant application) and application after the disease has set in (curative application).
3.10.3 Evaluation of efficacy of fungicides and bio-control agents on fusarium wilt when applied as curatives

Soil mixture was transferred into plastic pots at 1000 g per pot. The soil was artificially infested with *F. oxysporium* f. sp. *passiflorae* as explained in section 3.6 and left for 14 days before planting to allow the inoculums build up. The soil was then subjected to tests to determine the level of colonization by *Fusarium oxysporum* before transplanting seedlings; this was achieved by use of soil plate method where 5 g of soil was crushed in a sterile plate, adding 1ml of distilled water, followed by addition of molten Potato Dextrose Agar (PDA) and by determining the colony forming units on PDA after incubation for 3-5 days.

One 10 cm high tall (5 weeks old) seedling was then transplanted to each pot. Fertilizer (DAP, 18-46-0) was used at transplanting at the rate of 2 g per pot. Application of fungicides and bio-control agents was done two weeks after transplanting at the rate  Carbenadazim (0.01 ml per 12 ml of water per pot), Ridomil-Mz (0.03 g per 13 ml of water per pot), Thiophanate-methyl (0.01 per 12 ml of water per pot), Azoxystrobin (0.01 ml per 12 ml of water per pot) and Bio-control agents *T. harzianum* (0.5 g per 55 ml of water per pot) and *T. asperellum* (7.2 g per 55 ml of water per pot). The treatments (selected fungicides and bio-control agents) were applied as drench. Control pots were not treated. Each treatment was replicated three times and arranged in a completely randomized block design. Disease assessment was done in terms of disease severity described in section 3.8.1 and 3.8.2 while crop performance was assessed as described in section 3.9.
3.10.4 Evaluation of fungicides and bio control agents when applied as protectants

This experiment was carried out concurrently with curative. Treatments (fungicides and bio - control agent) were applied to sterile soil before inoculation procedures. Treatments were applied as per manufacturer’s recommendations. Inoculation of soil was done as explained in section 3.5. Control pots were without treatments. Each treatment was replicated three times and arranged in a completely randomized block design. Disease assessment was done as described in section 3.6.1 and 3.6.2. Crop performance (Shoot height, dry weight of roots and shoot) was assessed as described in section 3.7.

3.11 Data analysis

The data was subjected to analysis of variance (ANOVA) and means separated using Least Significance Difference (LSD) at a probability level of 95% (P ≤ 0.05) (Steel and Torie, 1982). Percentage values were transformed by getting their logarithm values to base 10 after which they were subjected to ANOVA. Genstat statistical package for Windows (Version 12) was used for the analysis.
CHAPTER FOUR

RESULTS

4.1 Effect of fungicides and bio-control agents on spore germination of *Fusarium oxysporum* f. sp. *passiflorae*

Laboratory assessment of four fungicides and two bio-control agents on spore germination of *F. oxysporum* f. sp. *passiflorae* show that there was significant (P<0.05) interaction between the treatments and time. The % means obtained indicate that the number of spore germination increased with time (12 hrs, 15 hrs and 18 hrs) (Table 4.1). Spore germination of *F. oxysporum* f. sp. *passiflorae* was effectively inhibited by Ridomil-Mz (Metalaxyl + mancozeb) compared to the other treatments with mean % of 21.46 ± 3.28, 31.10±1.15 and 31.79±2.03 from 12 hrs, 15hrs 18 hours respectively. However it’s interesting to note that *T. asperellum* also showed great abilities to inhibit spore germination of the pathogen. The control which had spores and sterile distilled waters had the highest spore germination with mean % of 84.83±1.59, 87.75±4.81, and 87.82±0.77 at 12 hrs 15 hrs and 18 hrs respectively after incubation (Table 4.1).

At 12 hrs Treatment with Ridomil-Mz *T. asperellum* and Carbendazim significantly (P<0.05) led to low germination of 21.46±3.28 %, 40.61±2.38 %, and 57.67±4.33 % respectively compared to Thiophanate-methyl (62.13±2.33 %) Azoxystrobin (51.39±1.39 %) and *T. harzianum* (71.11±1.39 %) (Table 4.1). At 15 hrs Ridomil-Mz and *T. asperellum* had significantly (P<0.05) higher effect on reducing the number of spore germination by 31.10±1.15 % and 43.33±4.41 respectively relative to Thiophanate-methyl (62.22±2.2 %) Azoxystrobin
(52.30±2.71 %) and T. harzianum (71.11±4.44 %). At 18 hrs Ridomil-Mz and T. asperellum significantly (p<0.05) reduced the spore germination by 31.79±2.38 % and 45.17±4.21 % respectively compared to other control agents (Table 4.1). There was no significant difference (P>0.05) observed when Thiophanate-methyl, Carbendazim, Azoxystrobin and T. harzianum at 18 hrs (Table 4.1). All the fungicides and bio-control agents significantly (p<0.05) reduced spore germination compared to control at all times (12 hrs, 15 hrs, 18 hrs).

Table 4.1: Spore germination (%) of Fusarium oxysporum f. sp. passiflorae treated with selected fungicides and bio-control agents after 12, 15 and 18 hours

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean % Germination</th>
<th>12 hrs</th>
<th>15 hrs</th>
<th>18 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td></td>
<td>62.13±2.23b**</td>
<td>62.22±2.22b</td>
<td>64.84±1.02b</td>
</tr>
<tr>
<td>Carbendazim</td>
<td></td>
<td>57.67±4.33c</td>
<td>63.06±1.94b</td>
<td>64.00±2.08b</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td></td>
<td>51.39±1.39b</td>
<td>52.30±2.71b</td>
<td>52.38±2.38b</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td></td>
<td>21.46±3.28c</td>
<td>31.10±1.15c</td>
<td>31.79±2.03bc</td>
</tr>
<tr>
<td>T. harzianum</td>
<td></td>
<td>71.11±4.44b</td>
<td>73.07±2.39b</td>
<td>74.56±10.01b</td>
</tr>
<tr>
<td>T. asperellum</td>
<td></td>
<td>40.61±2.38c</td>
<td>43.33±4.41c</td>
<td>45.17±4.21c</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>84.83±1.59a</td>
<td>87.75±4.81a</td>
<td>87.82±0.77a</td>
</tr>
</tbody>
</table>

*Means of 4 replicate
**Means followed by the same letter within the same column are not significantly different at P<0.05.

4.2 Effect of fungicides and bio-control agents on colony diameter of Fusarium oxysporum f. sp. passiflorae

The interaction between fusarium control agents and time of application on colony growth was significant (P<0.05). Carbendazim and Ridomil-Mz inhibited colony
diameter at 100 % level at all times (day 3 to day 7). There was no significant (P>0.05) difference in % inhibition between Carbendazim (100 % inhibition) and Ridomil-Mz (100 % inhibition) from day 3 to 7 (Table 4.2). The bio-control agent *T. asperellum* showed a similarly high level of colony inhibition which was 100 % inhibition the third day after incubation. The inhibition percentage of colony diameter by *T. asperellum* reduced from 100 % the third day to a mean inhibition percentage of 80.88 the seventh day although its effect remained significantly (P<0.05) higher compared to Azoxystrobin Thiophanate-methyl and *T. harzianum* (Table 4.2).

The colony inhibition % of *T. harzianum* and control did not differ significantly (P>0.05) on day 3 and day 4, after incubation, however, significant (P. < 0.05) difference between the two was observed on day 5, 6 and 7 after incubation where *T. harzianum* inhibited colony diameter by 7.53±4.94 % day 5, 31.95±2.57 % day 6, and 32.46±1.19 % day 7 significantly (P>0.05) compared to control which had colony diameter of 0.00 day 5, 0.00 % day 6, and 0.00 % day 7 (Table 4.2). Azoxystrobin significantly (P>0.05) produced higher inhibition percentages of 79.86±1.23 % and 76.31±3.03 % on day 3 and day 4 respectively compared to inhibition percentages of 70.08±1.35 % 67.85±1.35 % and 66.32±1.21 % obtained on 5, 6 and 7 day respectively after incubation (Table 4.2). Thiophanate-methyl significantly (P>0.05) produced higher inhibition percentages of 40.20±2.94 % on the third day compared to inhibition percentages produced on 4, 5, 6 and 7 day (Table 4.2). In general, Carbendazim and Ridomil-Mz were most effective
fungicides they inhibited colony diameter growth 100% throughout the incubation period followed by *T. aperellum*, and Azoxystrobin.

Thiophanate-methyl and *T. harzianum* did not significantly (P>0.05) differ in inhibition percentage on 6 and 7 day but the two significantly (P<0.05) differed in inhibition percentages on the 3, 4 and 5 day where Thiophanate-methyl produced higher percentages compared to *T. harzianum* (Table 4.2). Apart from *T. harzianum* which did not produce significant (P>0.05) difference on the 3, 4 and 5 day compared to control all other fungicides and *T. asperellum* significantly (P<0.05) inhibited colony growth compared to control (Table 4.2).
Table 4.2: Effect of fungicides and bio - control agents on percentage growth inhibition of colony diameter of *Fusarium oxysporum* f.sp. *passiflorae* from 3 to 7 day after inoculation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time in days</th>
<th>*Mean % inhibition of Colony</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Thiophanate-methyl</td>
<td>40.20±2.94\textsuperscript{f}</td>
<td>33.61±2.80\textsuperscript{g}</td>
<td>32.86±1.43\textsuperscript{g}</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>100±0.00\textsuperscript{a}</td>
<td>100±0.00\textsuperscript{a}</td>
<td>100±0.00\textsuperscript{a}</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>79.86±1.23\textsuperscript{cd**}</td>
<td>76.31±3.031\textsuperscript{d}</td>
<td>70.08±1.05\textsuperscript{c}</td>
</tr>
<tr>
<td>Ridomil –MZ</td>
<td>100±0.00\textsuperscript{a}</td>
<td>100±0.00\textsuperscript{a}</td>
<td>100±0.00\textsuperscript{a}</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>1.00±0.00\textsuperscript{i}</td>
<td>3.93±2.93\textsuperscript{hi}</td>
<td>7.53±4.94\textsuperscript{h}</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>100±0.87\textsuperscript{a}</td>
<td>82.29±1.26\textsuperscript{bc}</td>
<td>85.54±0.55\textsuperscript{b}</td>
</tr>
<tr>
<td>Control (Sterile water)</td>
<td>0.00±0.00\textsuperscript{i}</td>
<td>0.00±0.00\textsuperscript{i}</td>
<td>0.00±0.00\textsuperscript{i}</td>
</tr>
</tbody>
</table>

*Means of 4 replicates

**Means followed by the same letter within the same column and row are not significantly different at \( P \leq 0.05 \).
4.3 Effect of fungicides and bio-agents applied as curative and protectants on disease severity of passion fruit infected with *F. oxysporum f. sp. passiflorae*

In trial one the severity of *Fusarium* disease caused by *Fusarium oxysporum f. sp. passiflorae* on passion fruit was significantly (*P* < 0.05) affected by the interaction between the mode of application of the various fungicides and bio-control agents (Figure 4.1). Protectants had significantly (*P*<0.05) lower severity indices as compared to curatives. When control agents were applied as protectants Carbendazim had the least severity of (0.28) then Ridomil Mz (0.37) followed by *T. asperellum* (0.52) *T. harzianum* (0.67), Azoxystrobin (0.74) and Thiophanate-methyl (0.96).

When control agents were applied as curative Carbendazim (0.87) significantly (*P*<0.05) led to lower disease severity compared to other control agents. Carbendazim was followed *T. asperellum* (0.74) then *T. harzianum* (1.02) Thiophanate-Methyl (1.22) Ridomil Mz (1.59) and Azoxystrobin (1.61). There was no significant difference (*P*>0.05) observed when Azoxystrobin and Ridomil Mz were used under curative application where they both led to higher disease severity compared to other control agents. It’ is also important to note that Carbendazim showed consistency in reducing severity under both applications as protectant and as curative. The other control agents did not show this kind of consistency under both conditions. All control agents significantly (*P*<0.05) reduced severity as compared to the control (Figure 4.1) under both methods of application.
Figure 4.1: Effect of fungicides, bio-control and mode of application on severity of passion fruits infected with F. oxysporum f. sp. passiflorae (Trial 1).

In trial 2 the severity of fusarium disease caused by Fusarium oxysporum f. sp. passiflorae on passion fruit was significantly (P<0.05) affected by the interaction between the mode of application of the various fungicides and bio-control agents (Table 4.3). Protectants had significantly lower severity indices as compared to curatives. Passion fruit plants treated with Carbendazim and Ridomil-Mz significantly (P<0.05) led to lower severity under both protectant and curative applications. Carbendazim and Ridomil-Mz showed consistency in reducing disease severity under both application methods. Carbendazim produced higher severity of 0.83+0.09 when applied as curative as compared to protectant where severity was 0.56+0.11 application. Higher severity was observed under the treatment with T. asperellum in both applications. T. asperellum did not differ
significantly (P>0.05) from control when applied as curative but it significantly (P<0.05) reduced severity under protectant application compared to control. *Trichoderma harzianum* and *T. asperellum* significantly (P<0.05) reduced disease severity when applied as protectant although the former significantly (P<0.05) reduced severity when applied as curative compared to control. Apart from *T. asperellum* the rest of the control agents significantly (P<0.05) reduced the severity when applied as protectants and curatives compared to controls (Table 4.3).

**Table 4.3: Effect of fungicides, bio-control and mode of application on severity of passion fruits infected with *Fusarium oxysporum f. sp. passiflorae*. (Trial 2)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectant</th>
<th>Curative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td>1.02±0.15&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.57±0.15&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0.56±0.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.83±0.09&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.85±0.14&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.63±0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>0.82±0.14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.94±0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. hazianum</em></td>
<td>0.94±0.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.70±0.17&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>1.07±0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.22±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>1.98±0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.28±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P≤0.05.
4.4 Effect of fungicides and bio-control agents applied as protectant and curatives on fusarium wilt incidence (%) in passion fruit

In trial one, significant (P<0.05) difference was observed when selected fungicides and bio-control agents were applied as Protectants (Table 4.4). *T. asperellum* led to higher disease incidence of 50.00±5.77 % compared to other treatments. Azoxystrobin and Carbendazim applied as protectants significantly (P<0.05) reduced the disease incidence by 16.67±3.33 % and 20.00±5.77 % respectively compared to other control agents but there was no significant difference (P>0.05) between them. Thiophanate methyl (33.33±3.33 %), *T. harzianum* (33.33±3.33 %) and Ridomil-Mz ((33.33±3.33 %) applied as protectants did not significantly (P>0.05) differ in reducing disease incidence. All control agents applied as protectants significantly (P<0.05) reduced the disease incidence compared to control (Table 4.4).

When control agents were applied as curatives Azoxystrobin Carbendazim, Ridomil-Mz and *T. harzianum* did not significantly (P>0.05) differ in reducing the disease incidence which was 23.33±3.33 %, 36.67±3.33 %, 33.33±3.33 %, 33.33±3.33 % respectively however, they significantly (P<0.05) reduced the disease incidence effectively compared to *T. asperellum* (53.33±6.67 %). Azoxystrobin applied as curative significantly (P<0.05) reduced the disease incidence by 23.33±3.33 % compared to Thiophanate-methyl (46.67±3.33 %) and *T. asperellum* (53.33±6.67 %). All control agents applied as curatives significantly (P<0.05) reduced the disease incidence compared to controls (Table 4.4).
Table 4.4: Incidence (%) of fusarium wilt on passion fruits following application of fungicides and bio-control agents as protectants and curatives. (Trial 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>*Disease incidence (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protectant</td>
<td>Curative</td>
</tr>
<tr>
<td>Thiophanate-Methyl</td>
<td>33.33±3.33&lt;sup&gt;c&lt;/sup&gt;c</td>
<td>46.67±3.33&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>20.00±5.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.67±3.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>16.67±3.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.33±3.33&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>33.33±3.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.33±3.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>33.33±3.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.33±3.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>T. asperellum</td>
<td>50.00±5.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.33±6.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>66.67±3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.00±10.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Mean of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P≤0.05.

In the repeat trial (trial 2) the trend of disease incidence followed that of trial 1 except for the higher disease incidence in the later experiment. Significant (P<0.05) difference was observed when control agents were applied as protectants (Table 4.5) Azoxystrobin and Carbendazim applied as protectants significantly (P<0.05) reduced the percentage incidence by 26.67±3.33 % and 26.67±6.67 % respectively compared to other treatments. Although Azoxystrobin and Carbendazim did not significantly (P>0.05) differ in reducing the disease incidence when applied as protectants. T. asperellum applied as protectant led to higher disease incidence of 63.33±6.67 % compared to other treatments when applied as protectants. There was no significant (P>0.05) difference in disease incidence reduction among Thophanate-methyl (43.33±3.33 %) Ridomil Mz (43.33±3.33 %) and T. harzianum (40.00±5.77 %) when they were applied as
protectant. All Control agents significantly (P< 0.05) reduced the disease incidence under protective application compared to control. When control agents were applied as curative there was no significant (P>0.05) difference in disease incidence reduction amongst Azoxystrobin (33.33±3.33 %), Ridomil-Mz (43.33±3.33 %), T. harzianum (43.33±3.33 %) and Carbendazim (46.67±3.33 %) however, the four control agents significantly (P<0.05) reduced the incidence compared to control (90.00±10.00). T. asperellum led to higher disease of 63.33±6.67 % compared to other treatments when applied as curative. All the treatments when applied as curatives significantly (P<0.05) reduced the disease compared to controls. Just as trial 1 trial 2 protectants reduced disease incidence effectively compared to curatives (Table 4.5).

Table 4.5: Incidence (%) of fusarium wilt disease on passion fruits following application of fungicides and bio-control agents as protectants and as well as curatives. (Trial 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectants</th>
<th>Curative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td>43.33±3.33&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>56.67±3.33&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>26.67±6.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>46.67±3.33&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>26.67±3.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.33±3.33&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>43.33±3.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.33±3.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>40.00±5.77&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>43.33±3.33&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>T. asperellum</td>
<td>60.00±5.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.33±6.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>83.33±3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.00±10.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Mean of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P<0.05.
4.5 Effect of fungicides and bio-agents applied as curative and protectants on height of passion fruit infected with *F. oxysporum f. sp. passiflorae*

In trial one the height of passion fruit was significantly (P<0.05) affected by the method of application of fungicides and bio-control agents (Figure 4.2). When agents were applied as protectants *T. harzianum* led to highest height of 21.51 cm followed by Carbendazim (20.51 cm), Ridomil-Mz (19.8 cm) Thiophanate–methyl (18.93 cm). Lower heights were observed when *T. asperellum* (16.44 cm) and Azoxystrobin (17.51 cm) were applied as protectant though they were significantly (P<0.05) higher than the control heights of 16.65 cm. When the control agents were applied as curatives Carbendazim significantly (P<0.05) led to higher heights of 15.49 cm compared to other agents. Carbendazim was followed by Ridomil-Mz (13.68 cm) There was no significant (P>0.05) difference in height produced by Thiophanate-methyl (*T. asperellum, T. harzianum* and Azoxystrobin when applied as curatives. Agents applied as protectants led to significantly (P<0.05) higher heights than curatives in the first trial (Figure 4.2).
In the repeat trial (trial 2) the height of passion fruits was significantly \((P < 0.05)\) affected by the method of application of fungicides and bio-control agents (Figure 4.3). When the treatments were applied as protectants, Carbendazim produced higher heights of 16.75 cm followed by Thiophanate-methyl (13.5 cm) \(T. \) harzianum (12.9 cm) and Ridomil-Mz (11.64 cm). \(T. \) asperellum and Azoxystrobin produced lower heights of 11.95 cm and 11.15 cm respectively but the heights were significantly \((P<0.05)\) higher than the controls heights of 5.16 cm. Generally higher heights were recorded when agents were applied as protectants rather than curatives (Figure 4.3) although the heights of trial 2 were lower compared to heights of trial 1.
Effect of fungicides and bio-control agents on the length (cm) of root rot caused by *Fusarium oxysporum* f. sp. *passiflorae* on passion fruit

In the first trial when control agents were applied as protectants the shortest root rot length of 0.02±0.02 cm was observed when *T. asperellum* was used followed by Carbendazim and *T. harzianum* with a rot length of 0.50±0.26 cm and 0.58±0.44 cm respectively *T. harzianum* was followed by Thiophanate-methyl (1.00±0.67 cm) Ridomil-Mz (1.21±0.40 cm) and Azoxystrobin (1.57±0.53 cm). Although there was no significant differences (P>0.05) among all the control agents under protectant application (Table 4.6). All the control agents significantly (P<0.05) produced shorter root rot length compared to control when applied as protectants (Table 4.6).
When the control agents were applied as curatives Ridomil-Mz and *T. harzianum* led to significantly longer root rot length of 3.70±0.72 cm and 2.90 ±0.83 cm respectively. There was no significant difference (P>0.05) between Ridomil Mz and *T. harzianum*. Shorter root rot length of 1.22±0.41 cm, 1.30±0.25 cm, 1.42±0.40 cm and 1.43±0.49 cm was observed when Azoxystrobin Thiophanate – methyl *T. asperellum* and carbendazim were used respectively but there was no significant difference (P>0.05) among this control agents under curative application. Apart from Ridomil Mz and *T. harzianum* which did not differ significantly (P> 0.05) from control, all treatments applied as curatives significantly (P<0.05) had shorter root rot length compared to controls. Longer rot length were observed under curative application as compared to protectant application (Table 4.6).

Table 4.6: Effect of fungicides and bio-control agents on the length (cm) of rot caused by *Fusarium oxysporum* f. sp. *passiflorae* on passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Rot length (cm)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protectant</td>
</tr>
<tr>
<td>Thiophanate-Methyl</td>
<td>1.00±0.67bc**</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0.50±0.26bc</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>1.57±0.53bc</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>1.21±0.40bc</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>0.58±0.44bc</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>0.22±0.22c</td>
</tr>
<tr>
<td>Control</td>
<td>3.11±0.23a</td>
</tr>
</tbody>
</table>

*Means of 3 replicates

**Means followed by the same letter within the same column are not significantly different at P≤0.05.*
In the repeat trial (trial 2) when the control agents were applied as protectants Carbendazim and Thiophanate-methyl significantly (P<0.05) led to shorter root rot length of 0.20±0.05 cm and 0.27±0.06 cm respectively compared to other control agents under protectant application (Table 4.7). Azoxystrobin produced significantly different (P<0.05) longer root rot length compared to other control agents under protective application. There was no significant difference (P>0.05) in the rot length produced by *T. asperellum* (1.52±0.46 cm), *T. harzianum* (0.96±0.37 cm) and Ridomil-Mz (1.33±0.33 cm) under protective application. All control agents applied as protectants significantly (P<0.05) led to shorter rot length compared to the control which had highest rot length of 6.28±44 cm (Table 4.7).

When the control agents were applied as curatives Thiophanate-methyl and Carbendazim) significantly (P<0.05) reduced the rot by length by 0.4±0.08 cm and 0.82±0.08 cm respectively compared to other control agents (Table 4.7). Azoxystrobin when applied as curative had significantly (P<0.05) longer rot length of 4.00±0.25 cm compared to other control agents.

There was no significant difference (P > 0.05) in rot length produced by *T. asperellum* (1.73±0.35 cm) and *T. harzianum* (1.60±0.23 cm) but they both significantly (P<0.05) reduced the rot length compared to controls (7.89±0.42 cm) when they were applied as curatives. All the control agents applied as curatives significantly (P<0.05) led to shorter root rot length compared to controls (Table 4.7) Just as in trial one the protectants led to shorter root rot length compared to
curatives, although the rots were longer in the second trial under both application methods compared to the first trial.

**Table 4.7**: Effect of fungicides and bio-control agents on the length (cm) of root rot caused by *Fusarium oxysporum* f. sp. *passiflorae* on passion fruits (Trial 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectant</th>
<th>Curative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td>0.27±0.06d**</td>
<td>0.40±0.08e</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0.20±0.05d</td>
<td>0.82±0.08e</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>3.33±0.28b</td>
<td>4.00±0.25b</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>1.33±0.33c</td>
<td>2.92±0.24c</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>0.96±0.37c</td>
<td>1.60±0.23d</td>
</tr>
<tr>
<td>T. asperellum</td>
<td>1.52±0.46c</td>
<td>1.73±0.35d</td>
</tr>
<tr>
<td>Control</td>
<td>6.28±0.44a</td>
<td>7.89±0.42a</td>
</tr>
</tbody>
</table>

*Means of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P≤0.05.

### 4.7 Effect of fungicides and bio-control agents on shoot dry weight (g) of passion fruit infected with *Fusarium oxysporum* f. sp. *passiflorae*

In trial one the interaction between fusarium control agents and application method produced significant (P<0.05) differences on the dry weights of Passion fruit shoots (Figure 4.4). The dry weight of shoots obtained from passion fruits treated with different fusarium control agents was significantly (P<0.05) different. Passion fruit shoots treated with Ridomil-Mz significantly (P<0.05) produced the highest shoot dry weight of 5.17±0.03 g when applied as a protectant compared to other control agents. Carbendazim with a shoot dry weight of 3.63±0.33 g was second to
Ridomil-Mz in producing higher shoot weights. Azoxystrobin (2.45±0.52 g), Thiophanate- methyl *T. harzianum* (2.00±0.29 g) and *T. asperellum* (2.33±0.60 g) did not significantly (P>0.05) differ in shoot dry weight when applied as protectant. Ridomil-M significantly (P<0.05) had higher shoot dry weight of 5.17±0.33 g when applied as protectant compared to curative application where dry shoot weight was 2.39±0.49 g (Figure 4.4).

Thiophanate methyl significantly (P<0.05) had higher shoot dry weights of 2.17±0.167 cm when applied as protectants compared to curative application where it produced shoot dry weight of 0.57±0.16 cm. Thiophanate-methyl did not differ significantly (P>0.05) differ from controls in shoot dry weight when applied as curatives. All the control agents when applied as protectants significantly (P<0.05) had higher shoot dry weights compared to controls. Apart from Thiophanate-methyl the rest of the control agents significantly (P<0.05) had higher shoot dry weight compared to controls when applied as curatives. It’s also clear that the means of shoot dry weight obtained from protectants were higher than mean dry weights obtained from curative treatment (Figure 4.4).
Figure 4.4: Effect of fungicides, bio-control agents and mode of application on shoot dry weight (g) of passion fruits (Trial 1).

In the repeat trial (Trial 2) when the control agents were applied as protectants Carbendazim and Ridomil-Mz significantly (P>0.05) produced higher shoot dry weight of 2.65±0.45 g and 2.44±0.63 g respectively compared to T. harzianum (2.24±0.47 g), T. asperellum (1.38±0.56 g), Azoxystrobin (1.82±0.55 g) and Thiophanate –methyl (1.36±0.44 g) (Table 4.8). There were no significant (P>0.05) differences observed among the control agents applied as protectants. All the control agents produced higher means of dry weights when applied as
protectants compared to control. When the control agents applied as curatives Carbendazim, and Azoxystrobin significantly (P<0.05) led to higher shoot dry weights of 1.96±0.23 g and 1.92±0.38 g respectively compared to other control agents. There was no significant (P>0.05) difference among T. asperellum T. harzianum, Ridomil Mz and Thiophanate-methyl in dry shoot weights when they were applied as curatives. Apart from Thiophanate-methyl which did not significantly (P>0.05) differ from the control when applied as curative the rest of control agents significantly (P<0.05) had higher shoot dry weights compared to controls when applied as curatives. The dry shoot weight means of protectants were higher compared to curatives (Table 4.8).

Table 4.8: Effect of fungicides and bio-control agents applied as protectants and curatives on shoot dry weight of passion fruits in grams (Trial 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectant</th>
<th>Curative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td>1.36±0.44b</td>
<td>0.73±0.13cd*</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>2.65±0.45a</td>
<td>1.96±0.23a</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>1.82±0.55b</td>
<td>1.92±0.38a</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>2.44±0.63a</td>
<td>0.80±0.22bc</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>2.24±0.47ab</td>
<td>0.92±0.07bc</td>
</tr>
<tr>
<td>T. asperellum</td>
<td>1.38±0.56b</td>
<td>1.05±0.09bc</td>
</tr>
<tr>
<td>Control</td>
<td>0.33±0.067d</td>
<td>0.43±0.05d</td>
</tr>
</tbody>
</table>

*Means of 3 replicates.
**Means followed by the same letter within the same column are not significantly different at P≤0.05.
4.8 Effect of fungicides and bio-control agents on root dry weight (g) of passion fruit infected with *Fusarium oxysporum* f. sp. *passiflorae*

In trial one when control agents were applied as protectants Carbendazim significantly (P<0.05) had higher root dry weights of 0.88±0.10 g compared to other control agent (Table 4.9). Carbendazim was followed by Ridomil-Mz which significantly (P<0.05) led to higher root weight of 0.65±0.11 g compared to other control agents under protective application. Ridomil was followed by Thiophanate methyl, *T. asperellum*, *T. harzianum* and Azoxystrobin with root dry weight of 0.23±0.04 g, 0.42±0.01 g, 0.21±0.04 g and 0.23±0.05 g respectively. *T. asperellum*, *T. harzianum* and Azoxystrobin did not significantly (P>0.05) differ in root dry weights when applied as protectants. All control agents applied as protectants significantly (P<0.05) produced higher root weights compared to controls (Table 4.9).

When the control agents were applied as curatives Carbendazim significantly (P<0.05) had higher root weight of 1.05±0.18 g compared to the rest of the control agents. The second was Thiophanate-methyl which significantly (P<0.05) produced higher root dry weights of 0.56±0.12 g compared to Ridomil Mz *T. asperellum T. harzianum* and Azoxystrobin which had root dry weight of 0.36±0.1 g, 0.19±0.06 g, 0.14±0.06 g, 0.35+0.09 g respectively. Ridomil Mz (0.36+0.10 g), *T. asperellum* (0.19+0.06 g) *T. harzianum* (0.14+0.06 g) and Azoxystrobin significantly (P<0.05) produced high root dry weights compared to control when applied as curatives (Table 4.9).
Table 4.9: Effect of fungicides and bio-control agents applied as protectants and curative on root dry weight of passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>*Root dry weight (g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protectant</td>
<td>Curative</td>
</tr>
<tr>
<td>Thiophanate-Methyl</td>
<td>0.42+0.01&lt;sup&gt;c**&lt;/sup&gt;</td>
<td>0.56+0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0.88+0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05+0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.23+0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.35+0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>0.65+0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36+0.10&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>0.23+0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.14+0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>0.21+0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.19+0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.10+0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.12+0.06&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P≤0.05.

In repeat trial (Trial 2). When control agents were applied as protectants Carbendazim had the highest root weights of 0.76±0.06 g followed by *T. harzianum* (0.53±0.16 g), Thiophanate-methyl (0.50±0.08 g) Ridomil-Mz, (0.4.8±0.1 g), Azoxystrobin (0.40+0.10 g) and *T. asperellum* (0.33±0.09 g).(Table 4.10)

Although Carbendazim had higher weights it did not significantly (P>0.05) differ from the root weights produced by Thiophanate-methyl, Ridomil, *T. harzianum*. Carbendazim differed significantly (P<0.05) from Azoxystrobin and *T. asperellum*.when applied as a protectant. Both bio-control agents had lower root dry weights compared to fungicides under protectant application. The bio-control agents applied as protectants significantly (P<0.05) produced higher root weights.
compared to controls (Table 4.10). The overall mean root weight of protectants was higher compared to curatives.

Table 4.10: Effect of fungicides and bio-control applied as protectants and curative on root dry weight of passion fruits (Trial 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectant *</th>
<th>Curative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophanate-Methyl</td>
<td>0.50±0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.48±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0.76±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.40±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ridomil-Mz</td>
<td>0.48±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.53±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;T. harzianum&lt;/i&gt;</td>
<td>0.53±0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.20±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;T. asperellum&lt;/i&gt;</td>
<td>0.33±0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.17±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.10±0.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means of 3 replicates
**Means followed by the same letter within the same column are not significantly different at P<0.05.
CHAPTER FIVE
DISCUSSION CONCLUSION AND RECOMMENATION

5.1 Discussion

5.1.1 Effect of fungicides and bio-control agents on spore germination of *Fusarium oxysporum* f. sp. *passiflorae*

Low germination of *F. passiflorae* obtained from dishes treated with fungicide and bio-control agents indicated that fungicides and bio-control agents inhibited germination. The suppressive effect of Ridomil-Mz (Metalaxyl + mancozeb) on spores of *F. oxysporum* f. sp. *passiflorae* might be due to the existence of multisite action of this fungicide mixture (Wangichunge, 1998). This fungicide suppresses synthesis of nucleic acid (RNA) (KerrKennar, 1981) and also inactivates enzyme function (Wangichunge, 1998). Azoxystrobin which belongs to the group of Strobilurins is known to interfere with spore germination and germ tube development by inhibiting electron transport leading low energy production in the mitochondria (Koehle *et al.*, 2007).

*T. asperellum* may have succeeded in inhibiting spore germination of *F. oxysporum* f. sp. *passiflorae* possibly by producing toxins and secondary metabolites including Virudofungins, Trichothecenes, Peptaiboils, and 6-n—pentylyprone and hexahydrobenzopyran-5-one-2) that are suppressive (Harman, 1996). The spores of *F. oxysporum* f. sp. *passiflorae* may be more sensitive or more susceptible to toxins and secondary metabolites produced by *T. asperellum* compared to toxins and metabolites produced by *T. harzianum* this may require further investigation.
5.1.2 Effect of fungicides and bio-control agents on colony diameter of *Fusarium oxysporum* f. sp. *passiflorae*

The significantly (P<0.05) short colony diameters and high colony diameter inhibition % obtained from petridishes treated with fungicides and bio-control agent (*T. asperellum*) when compared to control indicate that the fungicide treatment and bio-control agent (*T. asperellum*) significantly inhibited growth of *F. oxysporum* f. sp. *passiflorae*. However, the relatively higher inhibitory effect of fungicides was associated with Ridomil-Mz and Carbendazim. Ridomil-Mz acts on mycelia by same mode of action as in spore inhibition.

Carbendazim which acts by interfering with nuclear division (Agrios, 2005) also completely inhibited mycelia growth from day 3 to day 7. Azoxytrobin whose mode of action is interference with energy production (respiration) by inhibition of electron transport at the site of quinol oxidation (McGrath, 2006) was able to inhibit mycelia growth but the level of inhibition was significantly lower compared to Ridomil-Mz and Carbendazim. *T. asperellum* was also effective in inhibiting mycelia growth of *F. oxysporum* f. sp. *passiflorae* from day 3 to day 7 compared to control. On the other hand, *T. harzianum* was not able to effectively retard growth significantly on day 3 to day 5 compared to control but it significantly (P<0.05) retarded growth on day 6 and day 7 compared to control. Its known that both *T. asperellum* and *T. harzianum* are mycoparasitic fungus and they produce hydrolytic enzymes (*ß*, 1-3 Glucanase, *ß*, 1-4 Glucanase and Chitinase, protease and xylanase) which lyses the mycelia of phytopathogenic fungi (Harman, 2000, Agrios, 2005; Jayalakshmi, 2009). Possible explanations for the difference in
retardation of mycelia inhibition by *T. asperellum* and *T. harzianum* could be that probably *T. harzianum* produces hydrolytic enzymes at a later stage of *F. oxysporum* f. sp. *passiflorae* mycelia development (Wanginjunge 1998). The effect of age (time) should not be underestimated, since age has been found to increase or decrease sensitivity of fungi to lytic enzymes (Kucuk *et al.*, 2007; Faheem *et al.*, 2010).

5.1.3 **Effect of fungicides and bio-control agents on fusarium wilt incidence, severity and root rot length of passion fruit**

When fungicides and bio-control agents were applied as protectants, they showed a promising effect in reducing the incidence of fusarium wilt than when applied as curatives. When control agent is applied before germination and establishment of *F. oxysporum* f. sp. *passiflorae*, then it is likely to be more effective than when applied after establishment of the pathogen (Agrios, 2005). A possible explanation for the bio-control agents (*T. asperellum* and *T. harzianum*) to reduce fusarium wilt incidence could be the bio-control leads to soil suppressiveness for *Fusarium oxysporum* by antagonizing the pathogen through production of antibiotics and lytic enzymes the bio-control agents also outcompete the phytopathogen for food, and space. *Trichoerma* are also known to parasitize the on phytopathogen (Adams, 1990; Jayalakshmi, 2009; Mohammad *et al.*, 2012). *Trichoderma* is able to produce toxic compounds with direct antimicrobial activity against phytopathogenic fungi. They (*Trichoderma*) generate fungal substances which are able to stimulate plant to produce its own defense metabolites that induce plant resistance against the phytopathogen (Jolanta, 2011).
The *Trichoderma* causes inactivation of the pathogens' enzymes involved in the infection sites on the root (Mohammad *et al.*, 2012). The bio-control agent *T. asperellum* and *T. harzianum* were effective in reducing the disease incidence compared to control possibly due to above combined stated reasons. It has to be noted that *T. harzianum* which did not perform well at inhibiting spore germination was effective at reducing disease incidence compared to *T. asperellum* probably different species within *Trichoderma* produce different amounts of antibiotics, lytic enzymes which are effective against *F. oxysporum* f. sp. *passiflorae* at different stages of development (Wojtkowiak-Gebarowska and Pietr, 2006). Azoxystrobin had the greatest impact in terms of reducing the incidence of fusarium wilt on passion fruit; however, this effectiveness was not discernible in the repeat trial. Since the experiment was repeated in the same greenhouse, it may be argued that possibly the environmental changes might have affected the impact of Azoxystrobin on *F. oxysporum* f. sp. *passiflorae* rendering the fungicide less effective. Carbendazim and Ridomil-Mz were able to significantly reduce disease incidence relative to control. The efficacy of the two fungicides in reducing the disease incidence could be attributed to direct impact of the fungicides on *F. oxysporum* f. sp. *passiflorae*.

The efficacy of fungicides and bio-control in controlling root rot caused by *F. oxysporum* f. sp. *passiflorae* in the greenhouse was indicated by their ability to reduce the length of the discoloured root tissue. The enhanced plant growth and reduced root rot infection by *F. oxysporum* f. sp. *passiflorae* with fungicide
treatment indicate that these fungicides suppressed fungal infectivity as compared to controls. The effect of fungicide treatments on root infection may be attributed to the direct impact of these compounds (Bokshi et al., 2003). The two bio-control agents *T. harzianum* and *T. asperellum* proved to be effective in reducing rotting of passion fruit caused by fusarium wilt. Indeed, both of them were more effective in this regard than two fungicides Azoxystrobin and Ridomil-Mz.

Minimal severity of fusarium wilt on passion fruit was observed when Carbendazim and Ridomil-Mz were applied but they were more effective when applied as protectants. The mode of action of these fungicides appears to favor them because of their combination. The two bio-control agents significantly reduced severity of the disease on passion fruits particularly in the first trial.

However, relatively high severity was registered under both treatments in the second trial when they were applied as curatives. It is known that *Trichoderma* secretes different lytic enzymes that limit plant pathogenic fungi (Yedidia *et al.*, 1999; Anand and Jayarama, 2009). These enzymes might have been acting differently with growth of both plant and pathogen thus producing such varying observations (Mohammad *et al.*, 2012). It is important to try and establish a trend in behavior of the bio-control agents in order to make practical recommendations for farmers.
5.1.4 Effect of fungicides and bio-control agents on height, shoot weight and root weight of passion fruit infected with fusarium wilt disease

In both trials, the growth of passion fruit was higher when control agents of *F. oxysporum* f. sp. *passiflorae* were applied as protectants. It is a fact that *T. harzianum* leads to increased growth of plants (Baker, 1989; Kleifeld and Chet, 1992; Bailey and Lumsden, 1998; Ramot *et al*., 2004). Possible explanations for *T. harzianum* to increase height shoot and dry weights could be control of minor pathogens leading to stronger, growth and nutrient uptake, solubilization of insoluble minor nutrients in soil and production of growth hormone (Kücük and Kivanc, 2003). *T. harzianum* may enhance plant growth by increasing the solubility of zinc, copper, iron and manganese ions, all plant nutrients with low solubility. *T. harzianum* also increases plant nitrogen efficiency (Kücük and Kivanc, 2003). *Trichoderma* solubilizes phosphate and micronutrients that could be made available to provide plant growth (Altomere *et al*., 1999; Inbar *et al*., 1994; Pandya *et al*., 2011).

Treated plants with *Trichoderma* produce substances which induce defense reactions against pathogen infection and help to take in more nutrients from soil by plant (Jolanta, 2011). In the present study, plants treated with *Trichoderma* exhibited more growth in terms of increase in heights. Increment in biomass (shoot and root dry weight), however, experiments did not give very high biomass but they were still significantly higher than control plants and this is consistent with the findings of Wojtkowiak-Gebarowska and Pietr (2006) among other workers.
On the other hand, the most consistent fungicides that led to higher biomass in Passion fruit were Carbendazim and Ridomil-Mz especially when applied as protectants. The high plant growth and increase in biomass associated with the two fungicides in the greenhouse could be ascribed to the ability of these fungicides to delay or impair the infection process of *F. oxysporum* f. sp. *passiflorae* (Wangichunge, 1998).

5.2 Conclusions

According to the research findings in this study, the following are the inferences.

1. The results reveal that Carbendazim and Ridomil-Mz are most effective when applied as protectant to manage fusarium wilt caused by *F. oxysporum* f. sp. *passiflorae* but in their absence then Azoxystrobin may be used.

2. *Trichoderma asperellum* and *T. harzianum* are effective reducing disease severity disease when applied as protectants but the level of reduction may not be equal to that obtained with chemical fungicides.

3. The efficacy of *T. harzianum* was lower at inhibiting spore germination and mycelia growth of *F. oxysporum* f. sp. *passiflorae* but more effective at reducing disease incidence while *T. asperellum* reversed these roles. Therefore, *Trichoderma* species should be selected depending on the stage of development of the pathogen for effective management of fusarium wilt in passion fruit.
4. In light of the need to develop an environmentally friendly integrated pest management practice use of Carbendazim and Ridomil-Mz as well as the two *Trichoderma* species (*T. asperellum* and *T. harzianum*) should be incorporated in the management of fusarium wilt of passion fruits in Kenya but not forgetting that other cultural practices should be highly considered in order to achieve the best results.

5.3 **Recommendations**

1. Due to potential of bio-control agents (*T. harzianum* and *T. asperellum*) there is need to popularize its use in disease control programs.

2. Additional tests should be carried in the field to confirm efficacy of fungicides and *T. harzianum* and *T. asperellum* used in this research.

3. Carbendazim and Ridomil –Mz should be used as protectant fungicides in management of fusarium wilt in passion fruits.
REFERENCES


APPENDICES

Appendix 1: Spore germination (%) of *Fusarium oxysporum* f. sp. *passiflorae* treated with different fungicides and bio-control agents after 12, 15 and 18 hours

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>21.85</td>
<td>10.92</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>305.89</td>
<td>152.95</td>
<td>3.92</td>
<td>0.028</td>
</tr>
<tr>
<td>Treat</td>
<td>6</td>
<td>20741.3</td>
<td>3456.87</td>
<td>88.58</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time.Treat</td>
<td>12</td>
<td>178.32</td>
<td>14.86</td>
<td>0.38</td>
<td>0.963</td>
</tr>
<tr>
<td>Residual</td>
<td>40</td>
<td>1560.99</td>
<td>39.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>22808.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 2: Effect of fungicides and bio-control agents on mean mycelia inhibition% of *Fusarium oxysporum* f. sp. *passiflorae* from 3 to 7 day after inoculation

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>3</td>
<td>47.82</td>
<td>15.94</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>287.02</td>
<td>71.75</td>
<td>7.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Treat</td>
<td>6</td>
<td>200748</td>
<td>33458</td>
<td>3342.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time.Treat</td>
<td>24</td>
<td>5397.08</td>
<td>224.88</td>
<td>22.47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>102</td>
<td>1021</td>
<td>10.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>207500.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3: Incidence (%) of fusarium wilt on Passion fruits following application of fungicides and bio-control agents as curatives (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>295.24</td>
<td>147.62</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>6295.24</td>
<td>1049.21</td>
<td>13.92</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>12</td>
<td>904.76</td>
<td>75.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>7495.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 4: Incidence (%) of fusarium wilt on Passion fruits following application of fungicides and bio-control agents as protectants (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>123.81</td>
<td>61.9</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>5361.9</td>
<td>893.65</td>
<td>17.59</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>12</td>
<td>609.52</td>
<td>50.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>6095.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Appendix 5: Incidence (%) of fusarium wilt on Passion fruits following application of fungicides and bio-control agents as protectants (Trial 2)**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>66.67</td>
<td>33.33</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>7161.9</td>
<td>1193.65</td>
<td>16.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>12</td>
<td>866.67</td>
<td>72.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>8095.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Appendix 6: Incidence (%) of fusarium wilt on Passion fruits following application of fungicides and bio-control agents as curative (Trial 2)**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>295.24</td>
<td>147.62</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>6295.24</td>
<td>1049.21</td>
<td>13.92</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>12</td>
<td>904.76</td>
<td>75.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>7495.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 7: Effect of fungicides and bio-control agents applied as protectants on root dry weight of Passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>1.44451</td>
<td>0.24075</td>
<td>20.67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.16307</td>
<td>0.01165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>1.60758</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 8: Effect of fungicides and bio-control agents applied as curative on root dry weight of Passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>1.9161</td>
<td>0.31935</td>
<td>9.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.47313</td>
<td>0.0338</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>2.38923</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 9: Effect of fungicides and bio-control applied as protectants on root dry weight of Passion fruits (Trial 2)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>0.74626</td>
<td>0.12438</td>
<td>4.41</td>
<td>0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.39447</td>
<td>0.02818</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>1.14072</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 10: Effect of fungicides and bio-control applied as curative on root dry weight of Passion fruits (Trial 2)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>0.47059</td>
<td>0.07843</td>
<td>5.77</td>
<td>0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>0.19033</td>
<td>0.0136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>0.66092</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 11: Effect of fungicides and bio-control agents applied as protectants on the length (cm) of rot caused by *Fusarium oxysporum* *f. sp. passiflorae* on Passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>7.842</td>
<td>3.921</td>
<td>2.55</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>50.884</td>
<td>8.481</td>
<td>5.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>54</td>
<td>83.007</td>
<td>1.537</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>141.733</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 12: Effect of fungicides and bio-control agents applied as curative on the length (cm) of rot caused by *Fusarium oxysporum* *f. sp. passiflorae* on Passion fruits (Trial 1)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep stratum</td>
<td>2</td>
<td>22.616</td>
<td>11.308</td>
<td>4.85</td>
<td></td>
</tr>
<tr>
<td>Rep.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>84.279</td>
<td>14.046</td>
<td>6.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>54</td>
<td>125.896</td>
<td>2.331</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>232.79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 13: Effect of fungicides and bio-control agents applied as protectants on shoot dry weight of Passion fruits in grams (Trial 2)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>9.912</td>
<td>1.652</td>
<td>2.36</td>
<td>0.087</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>9.7837</td>
<td>0.6988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>19.6957</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 14: Effect of fungicides and bio-control agents applied as curatives on shoot dry weight of Passion fruits in grams (Trial 2)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>12.693</td>
<td>2.1155</td>
<td>4.63</td>
<td>0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>35</td>
<td>16.0013</td>
<td></td>
<td>0.4572</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>28.6943</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>