ANTAGONISTIC POTENTIAL OF LOCAL ENDOPHYTIC FUNGI AGAINST ROOT-KNOT NEMATODES (*Meloidogyne* spp.) ON TREE TOMATO (*Solanum betaceum*) IN NYANDARUA COUNTY, KENYA

WASWA STANLOUS JUMA (MSc. Crop Prot. - Plant Path.)

A99/21198/2021

A thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Plant Pathology in the School of Agriculture and Environmental Sciences, Kenyatta University

November, 2025

DECLARATION

I, Waswa Stanlous Juma, declare that this thesis is my original work and it has not been submitted for a degree or any other award at any other university or institution of higher learning.

Sign: .... Date: 24-11-2025

Waswa Stanlous Juma: Reg. No: A99/21198/2021

Department of Agricultural Sciences and Technology, Kenyatta University

Declaration by supervisors

We confirm that the work presented in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors.

Sign: .... Date: 24th Nov. 2025

Prof. Waceke Wanjohi

Department of Agricultural Sciences and Technology, Kenyatta University

Sign: .... Date: 24/11/2025

Prof. Maina Mwangi

Department of Agricultural Sciences and Technology, Kenyatta University

DEDICATION

This research work is dedicated to God for enabling me in terms of time, good health and finances to come this far. In addition, I dedicate this work to my family for their love, support, and understanding throughout my research.

ACKNOWLEDGEMENT

I want to most unreservedly thank my supervisors, Prof. Waceke Wanjohi and Prof. Maina Mwangi for their scholarly contributions, constructive remarks, patience, timeliness and guidance on this work. Their prompt responses and input have been amazing despite the busy offices they hold. My gratitude also goes to Prof. Maina Mwangi for his financial assistance that helped me buy a DNA extraction kit for molecular work. My sincere gratitude goes to both of them for facilitating me to get permission, space and access to chemicals and equipment to do molecular work in the Plant Transformation Laboratory in the Department of Biochemistry, Biotechnology and Microbiology at Kenyatta University. I am also indebted to Professor Steve Runo for giving me the chance to work on the molecular aspects of this study in the Plant Transformation laboratory. I would also wish to register my gratitude to Andrew Nuwamanya for his logistical and intellectual support regarding to molecular work. My sincere thanks also go to Madam Lucy Muchiri for her logistical assistance in the agiculture lab while not forgetting Mr. Victor Ngaiza and Mr. Simon Nyandwi for their good company and constructive engagements throughout this study. My heartfelt gratitude also goes to Mourine Mutai for her tremendous help on the analysis of the molecular work. I would also like to most unreservedly thank the Kenyatta University Plant Pathology students' platform spearheaded by Madam Lucy Muchiri for the many chances I was given to make mock presentations and their constructive criticism and comments on my work. Finally, my gratitude also goes to farmers in Nyandarua county for allowing me to gather samples from their farms and for giving me the opportunity to use their farms to conduct the field experiments.

TABLE OF CONTENTS

TITLE PAGE……………………………………………………………………..I

DECLARATION II

DEDICATION III

ACKNOWLEDGEMENT IV

TABLE OF CONTENTS V

LIST OF TABLES...X

LIST OF FIGURES XIII

LIST OF PLATES XV

LIST OF APPENDICES XVI

ABBREVIATIONS AND ACRONYMS XVII

ABSTRACT XVIII

CHAPTER ONE: INTRODUCTION...1

1.1 Background information 1

1.2 Statement of the research problem.. 3

1.3 Justification of the study 4

1.4 Objectives 4

1.4.1 General objective 4

1.4.2 Specific objectives 5

1.5 Hypotheses 5

1.6 Significance of the study 6

1.7 Conceptual framework 7

CHAPTER TWO: LITERATURE REVIEW 8

2.1 Origin and economic importance of tree tomato 8

2.2 Nutritional importance of tree tomato 9

2.3 Production of tree tomato in Kenya 9

2.4 Tree tomato production constraints in Kenya 10

2.5 Root-knot nematode damage to plants 11

2.6 General life cycle of root-knot nematodes 12

2.7 Management of root-knot nematodes 13

2.7.1 Use of crop rotation strategy 14

2.7.2 Use of resistant cultivars 15

2.7.3 Use of soil organic amendments 15

2.7.4 Use of biological control agents 16

2.7.5 Integrated root-knot nematode management 20

2.7.6 Use of chemical control strategy 21

CHAPTER THREE: MATERIALS AND METHODS 23

3.1 Sampling and experimental field sites 23

3.2 General methodologies 25

3.2.1 Sampling of tree tomato roots for extraction of endophytic fungi 25

3.2.2 Isolation of endophytic fungi from tree tomato roots..26

3.2.3 Isolation of endophytic fungi from *Meloidogyn*e eggs......................................27

3.2.4 Preparation of fungal inoculum 28

3.2.5 Preparation of root-knot nematode inoculum..29

3.2.6 Nursery establishment 30

3.2.7 Preparation of growth media for greenhouse experiments................................30

3.2.8 Seedbed preparation for field experiments...30

3.2.9 Evaluation of root-knot nematode disease parameters......................................31

3.2.10 Evaluation of plant growth parameters..33

3.3 Specific methodologies 33

3.3.1 Efficacy of endophytic fungi on mortality of RKN J2s *in-vitro* 33

3.3.2 Identification of endophytic fungi causing mortality of RKN J2s 34

3.3.2.1 Morphological identification of endophytic fungi 34

3.3.3 Compatibility of Velum® Prime with selected endophytic fungus 37

3.3.4 Efficacy of endophytic fungus combined with *Lantana camara* leaf extract and Velum® Prime against root-knot nematodes...40

3.4 Data analysis 45

CHAPTER FOUR: RESULTS...46

4.1 Efficacy of endophytic fungal isolates on mortality of RKN J2s 46

4.2 Identification of endophytic fungal isolates...51

4.2.1 Morphological descriptions of fungal conidia 53

4.2.2 Molecular identification of endophytic fungi from tree tomato 59

4.3 Compatibility of Velum® Prime with endophytic *Colletotrichum nigrum* and *Trichoderma asperellum* isolates against J2s of RKN on tree tomato 67

4.3.1 Effect of Velum® Prime on colony growth of *Colletotrichum nigrum* and commercial *Trichoderma asperellum in vitro* 67

4.3.2 Efficacy of *Colletotrichum nigrum* and *Trichoderma asperellum* combined with Velum® Prime against RKNs on tree tomato in the greenhouse 68

4.4 Efficacy of combining *Colletotrichum nigrum*, *Lantana camara* leaf extract and Velum® Prime against Root-knot nematodes on tree tomato 79

4.4.1 Effect of *Lantana* leaf extract on mortality of RKN J2s *in vitro* 79

4.4.2 Effect of combining *Lantana* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in vitro* 83

4.4.3 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime against RKNs on tree tomato in the greenhouse tests 86

4.4.4 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime against RKNs on tree tomato in the field, season I and II at Njabini and Engineer respectively 97

CHAPTER FIVE: DISCUSSION 105

5.1 Efficacy of endophytic fungal isolates on mortality of RKN J2s 105

5.2 Endophytic fungi of tree tomato from Nyandarua County 106

5.3 Compatibility of Velum® prime with endophytic *Colletotrichum nigrum* and commercial *Trichoderma asperellum* against RKNs on tree tomato 109

5.4 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana camara* leaf extract and Velum® Prime against root-knot nematodes on tree tomato 111

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS 118

6.1 Conclusions 118

6.2 Recommendations 119

REFERENCES 120

APPENDICES 140

LIST OF TABLES

Table 3.1: Scoring scale for root-knot nematode galling and egg mass indices 32

Table 4.1: Efficacy of endophytic fungal isolates on mortality (%) of RKN J2s in *in-vitro* test I... 47

Table 4.2: Efficacy of endophytic fungal isolates on mortality (%) of RKN J2s in *in-vitro* test II 48

Table 4.3: Conidia characteristics of fungal isolates 54

Table 4.4: GeneBank accession numbers obtained for isolates from this study and NCBI Accession reference numbers used in identification of species from ITS sequences 61

Table 4.5: Nucleotide frequencies 65

Table 4.6: Neutrality (Tajma's) test for endophytic fungal sequences 66

Table 4.7: Substitution of nucleotides 66

Table 4.8: Effect of Velum® Prime on *Colletotrichum nigrum* and *Trichoderma asperellum* growth (colony diameter) 67

Table 4.9: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test I................. 70

Table 4.10: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test II 71

Table 4.11: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on indices of galling and egg masses on tree tomato in the greenhouse test I and II........................... 73

Table 4.12: Shoot height (SH), dry root weight (DRW) and dry shoot weight (DSW) of tree tomato treated with *Colletotrichum nigrum* and *Trichoderma asperellum* combined with Velum® Prime in the greenhouse test I 77

Table 4.13: Shoot height (SH), dry root weight (DRW) and dry shoot weight (DSW) of tree tomato treated with *Colletotrichum nigrum* and

Trichoderma asperellum combined with Velum® Prime in the greenhouse test II 78
Table 4.14: Effect of varying concentrations of leaf extract of *Lantana camara* on mortality of RKN J2s *in-vitro* 80
Table 4.15: Efficacy of combining *Lantana camara* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in-vitro* 84
Table 4.16: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on RKN J2 populations and reproduction factor on tree tomato in the greenhouse test I (March- June 2023) 88
Table 4.17: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test II (Aug – Nov 2023) 89
Table 4.18: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on indices of galls and egg masses on tree tomato in the greenhouse tests (March- June, 2023) 92
Table 4.19: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height and dry weights of tree tomato in the greenhouse test I (March- June, 2023) 95
Table 4.20: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height and dry weights of tree tomato in the greenhouse test II (August – November, 2023) 96
Table 4.21: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the field season I (March- June, 2023) 98

Table 4.22: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the field season II (August - November, 2023)..99

Table 4.23: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on indices of galling and egg masses on tree tomato in the field (season I - March - June; season II - Aug - Nov. 2023) 101

Table 4.24: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height (SH); dry root weights (DRW) and dry shoot weight (DSW) of tree tomato in the field season I (March- June, 2023)....................103

Table 4.25: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height (SH); dry root weights (DRW) and dry shoot weight (DSW) of tree tomato in the field season II (August - November, 2023) 104

LIST OF FIGURES

Figure 1:1: Conceptual framework 7
Figure 2:1: Root-knot nematode disease cycle 13
Figure 3:1 Map of Nyandarua county showing Kinangop where sampling and field experiments were conducted. 24
Figure 4:1: Mean mortality of J2s at different times of exposure to fungal endophytes *in vitro* 50
Figure 4:2: Relationship between time of exposure and mortality of J2s *in vitro* 51
Figure 4:3: Distribution of endophytic fungi isolated from roots of tree tomato 52
Figure 4:4: Occurrence of endophytic fungi from eggs of RKNs on tree tomato 53
Figure 4:5: Gel electrophoresis of amplified PCR for ITS region of endophytic fungi 60
Figure 4:6: Neighbor-Joining phylogenetic tree indicating the relationship between endophytic fungal based on ITS sequences. 64
Figure 4:7: Regression analysis between nematode reproduction factor and populations of J2s in the soil (Greenhouse experiment) 72
Figure 4:8: A line graph showing the relationship between time of exposure to *Lantana* leaf extract and mortality of J2s *in vitro* 81
Figure 4:9: Line graphs showing the relationship between various concentrations of *Lantana* leaf extract and mortality of J2s *in vitro* 82
Figure 4:10: Relationship between the concentration of *Lantana* leaf extract and mortality of J2s after exposure for 72 hours *in vitro* 83
Figure 4:11: Effect of varying concentration of *Lantana* leaf extract combined with *C. nigrum* on mortality of J2s *in vitro* 85
Figure 4:12: Relationship between the time of exposure to various concentrations of *Lantana* leaf extract combined with *C. nigrum* in *in vitro* test 86
Figure 4:13: Regression analysis between nematode reproduction factor and population of J2s in the soil (Greenhouse test) 90

Figure 4:14: Regression analysis between nematode reproduction factor and population of J2s in the soil (Field expt.) 100

LIST OF PLATES

Plate 4.1: Appearance of RKN second stage juveniles after exposure to endophytic fungi *in-vitro* 49

Plate 4.2: Conidia of fungal isolates 59

Plate 4.3: Effect of Velum® Prime on colony diameter of endophytic *Colletotricum nigrum* and commercial *Trichoderma asperellum* 68

Plate 4.4: Differences in growth of tree tomato under different treatments in the greenhouse. 75

Plate 4.5: Effect of root-knot nematode infestation on indices of galling and egg masses on roots of tree tomato in the greenhouse test. 91

LIST OF APPENDICES

Appendix 1: Turn-it-in report summary 140

Appendix 2: Research publications and conference presentation 141

Appendix 3: Research authorization 143

Appendix 4: Approval of research proposal letter 144

Appendix 5: Research permit 145

Appendix 6: Morphological descriptions of endophytic fungal isolates 146

Appendix 7: ITS sequences for 18 endophytic fungi of tree tomato 151

ABBREVIATIONS AND ACRONYMS

ai	Active ingredient
ANOVA	Analysis of Variance
BCAs	Biological Control Agents
DNA	Deoxyribonucleic acid
EF	Endophytic Fungi
INM	Integrated nematode management
IPM	Integrated Pest Management
J2	Infective second stage juveniles of root-knot nematodes
KES	Kenya Shillings
PCR	Polymerase Chain Reaction
PPNs	Plant Parasitic Nematodes
RKNs	Root-Knot Nematodes

ABSTRACT

Tree tomato (*Solanum betaceum* Cav.) is a significant emerging horticultural cash crop in Kenya with an estimated economic value of over KES 0.5 billion and has many nutritional and health benefits. Root-knot nematode (RKNs) infestations which results in significant yield losses, pose a threat to tree tomato growing in Kenya. Chemical nematicides have been the main method of controlling RKNs. However, chemical nematicides can be harmful to the environment and human health. Thus, safer alternatives to current techniques of management are needed. The study's objectives were: (i) to determine effects of the endophytic fungi associated with tree tomato roots on second stage juveniles (J2s) of RKN *in vitro*; (ii) to characterize endophytic fungi with potential against J2s of RKN; (iii) to ascertain whether Velum® Prime SC 500 (ai = fluopyram 500g/L) is compatible with endophytic fungi *in vitro* and under greenhouse conditions, and (iv) to assess the effectiveness of combining Velum® Prime, *Lantana camara* L. leaf extract, and the most effective endophytic fungus (*Colletotrichum nigrum*) against RKNs on tree tomatoes in both *in vitro* and *in vivo* settings. The experiments were laid down in completely randomized and randomized complete block designs in the greenhouse and in the field, respectively, with four replicates per treatment and terminated 90 days after transplanting. Data on initial and final nematode populations, total number of RKN J2s, the galling and egg mass indices, the nematode reproduction factor, height of shoot, and weights of dry roots and shoots were all recorded. The data were analysed using ANOVA with SAS software version 9.2, and significant means were separated using Tukey's Honest Significant Difference test at a 5% level ($P \leq 0.05$). Regression and correlation analyses were performed to ascertain the connections between RKN disease parameters. Forty four endophytic fungi isolated from tree tomato roots belonged to nine genera (*Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp., *Penicillium* sp., *Didymella* sp., *Periconia* sp., *Microsphaeropsis* sp., *Rhizoctonia* sp. and *Purpureocillium* sp.). *Fusarium* sp. (37%) and *Purpureocillium* sp. (34%) were most abundant in roots and eggs of RKNs respectively. *Colletotrichum nigrum* caused the highest mortality of J2s of 87 % followed by *Aspergillus ustus* and *Fusarium solani* with 63.5% each *in-vitro*. Velum® Prime did not inhibit colony growth of *C. nigrum* on PDA media. *Colletotrichum nigrum* combined with Velum® Prime significantly reduced J2s in soil and nematode reproduction factor in the greenhouse and were therefore compatible. Undiluted *Lantana* leaf extract + 1×10^6 *C. nigrum* caused mortality of J2s with 81% and 83 % in tests I and II, *in-vitro*. *Colletotrichum nigrum* + *Lantana* leaf extract + Velum® Prime significantly reduced plant disease parameters (J2s, gall and egg mass indices and nematode reproduction) and also increased plant growth parameters (shoot height and dry weights) in the greenhouse and in the field compared to control. New knowledge on integrated nematode management using endophytic fungi and *Lantana* leaf extract against second stage juveniles of RKNs will help farmers in adopting affordable and eco-friendly methods to improve tree tomato production and increase yield. There is need to commercialize *C. nigrum* product and promote use of safe strategies to manage RKNs.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Tree tomato, (*Solanum betaceum* Cav.), is a significant horticultural crop in Kenya and globally (Muriithi *et al*., 2013; Ramírez, 2017). It has a significant economic benefit (AFA, 2021) for smallholder farmers in Kenya since it improves food security and their standard of living through income generation (Muriithi *et al*., 2013). Tree tomatoes are becoming increasingly appreciated as an economic crop with nutritional and health benefits (AFA, 2021). When ripe, the fruits are consumed raw or turned into juice. Iron, phosphorus, potassium, calcium, antioxidants, and vitamins A, B, C, and E are abundant in the fruits (Pedrosa, 2016).

Tree tomatoes are parasitized by RKNs (*Meloidogyne* spp.), fungi, bacteria and viruses which limit production (Prohens and Neuz, 2001). There are 90 known species of *Meloidogyne* spp. with *M. javanica*, *M. arenaria*, *M. incognita*, and *M. hapla* being the most significant for agriculture. It is predicted that RKNs cause lose of up to USD 80 billion annually in agricultural damage worldwide (Navia *et al*., 2017). Root-knot nematodes attack wide variety of plant hosts with vast distribution across the world and form disease complexes with other plant pathogens (Jones *et al*., 2013). Root-knot nematodes attack tree tomatoes reducing their yields in many parts of Kiambu and Embu counties in Kenya (Waswa *et al*., 2020). The actual extent of damage has not been documented but it could be more than 30% (Sikora and Fernandez, 2005).

One of Kenya's top tree tomato growing areas is Nyandarua County (AFA, 2021). The County's tree tomato production, area and value, as of 2019 was 9,479

metric tons, 316Ha and 4.71 Million USD (KES. 658.9 million), respectively, while in 2020 it was 9,525 tons, 318Ha, and 4.73 Million USD (KES. 661.5 million), respectively, translating into 55.1 % of the overall fruit production value in the county (AFA, 2021).

Chemical nematicides, physical means, cultural approaches, resistant cultivars, and biological control are some of the methods used in the management of *Meloidogyne* spp. Chemical nematicides are considered toxic to humans, detrimental to beneficial microorganisms, expensive, and lose their effectiveness with extended usage (Onkendi *et al*., 2014). Finding a control strategy that is safe for users, efficient and environmentally beneficial is therefore necessary.

Since RKNs are sedentary endoparasites, rhizosphere soil microorganisms are unable to effectively regulate them. One low-cost and environmentally benign way to manage RKNs is to use local fungal endophytes (Oono *et al*., 2015; Potshangbam *et al*., 2017). Plant development is stimulated by *Fusarium* spp., whereas plant pathogenic nematodes are suppressed by *Trichoderma* spp. (Oono *et a*l., 2015; Potshangbam *et al*., 2017). Most agricultural crops are colonized by local endophytic fungal antagonists, which are becoming more and more well-known as possible antagonists against plant parasitic nematodes (Oono *et al*., 2015; Potshangbam *et al*., 2017).

Consequently, to manage RKNs in tree tomatoes, it is necessary to determine local endophytic fungi connected with plant roots and with *Meloidogyne* eggs. This study aimed at identifying endophytic fungi (EF) associated with tree tomatoes and assesses their effectiveness against RKN on a susceptible tree tomato cultivar (Goldmine) when applied in combination with *Lantana camara* L. leaf extract.

1.2 Statement of the research problem

Root-knot nematodes are polyphagous and prominent plant parasitic nematode (PPN) that damage most agricultural crops globally. The RKNs have been extracted from tree tomato roots in many areas of Kiambu and Embu counties. Their occurrence seriously damages the roots (Waswa *et al*., 2021) thereby affecting plant growth and performance. The current management strategies have not been effective. Since the infective RKNs (J2s) live in the soil and in roots of plants, generate symptoms akin to those of mineral and water deficits, they have short live cycles and hence they are more difficult to control than other pathogens (Jones *et al*., 2013; Chen *et al*., 2020). *Meloidogyne* species are the most devastating to crops and are among the topmost ten primary plant parasitic genera globally (Jones *et al*., 2013; Chen *et al*., 2020). Root-knot nematodes pierce and penetrate roots of plants which serve as ports of entry for other pathogenic microbes that form diseases complexes which compounds the losses. As such, they represent a significant risk to the production of tree tomatoes. It's becoming more crucial to manage RKNs using environmentally friendly alternatives because of the growing worries about chemical usage and its consequences on human health and the surrounding environment. Therefore, use of biological control and integrated nematode management approaches can offer alternative methods that are not hazardous as compared to chemicals such as aldicarb and carbofuran which are currently widely used by farmers. Research on the biological control and integrated management of RKNs in tree tomato using locally available endophytic fungi has not been done. It is therefore necessary to explore local endophytic fungi species for antagonism to RKNs on tree tomato and test their efficacy in integrated nematode management.

1.3 Justification of the study

The approach of using endophytic fungi in suitable integrated RKN management systems has the potential to enhance tree tomato production in Kenya. This is because fungal endophytes and RKNs inhabit the same ecological niche. As a result, using endophytic fungi to manage RKNs may be a practical and environmentally beneficial solution. Furthermore, there has not been any research or documentation on the combined use of botanical extracts from *Lantana* and fungal endophytes to manage RKNs on tree tomatoes in Kenya. It is therefore necessary to assess the endophytic fungi associated with tree tomatoes and identify the best isolate to combat RKNs. Fungal endophytes and *Lantana camara* L. leaf extract are environmentally beneficial, they have no risk to human health, they reduce nematode damage, they are economical to use and hence reduces the production costs. Tree tomato farming is a lucrative venture that generates income to farmers through fruit sales. Managing RKNs using endophytic fungi and *Lantana* leaf extract will boost production, increase the sales and improve rural livelihoods. Tree tomato growers will have a safer alternative way to manage RKNs by utilizing integrated nematode management (INM), which combines the use of fungal antagonists with *Lantana* leaf extract and Velum® Prime.

1.4 Objectives

1.4.1 General objective

To enhance tree tomato yield and productivity through sustainable integrated management of RKNs.

1.4.2 Specific objectives

i. To determine the effects of endophytic fungi associated with tree tomato roots and eggs of RKNs on mortality of RKN second stage juveniles (J2s) *in vitro*.

ii. To characterize local endophytic fungi that are effective on RKN J2s (in i above) in Nyandarua County.

iii. To determine the compatibility of a synthetic nematicide (Velum® Prime) with the most effective endophytic fungus against J2s of RKN *in vitro* and in the greenhouse.

iv. To evaluate the efficacy of combining the most effective endophytic fungus with *Lantana camara* leaf extract and Velum® Prime against RKN second stage juveniles on tree tomato *in vitro* and *in vivo*.

1.5 Hypotheses

i. Endophytic fungi isolated from tree tomato roots do not cause significant mortality of RKN second stage juveniles *in vitro*.

ii. Fungal endophytes associated with tree tomato roots and eggs of RKN in Nyandarua County are not culturally, morphologically and genetically diverse.

iii. Velum® Prime combined with selected endophytic fungus does not significantly suppress RKN second stage juveniles on tree tomato *in vitro* and in the greenhouse.

iv. Combined application of selected endophytic fungus, *Lantana camara* leaf extract and Velum® Prime does not significantly suppress RKN second stage juveniles on tree tomato *in vitro* and *in vivo*.

1.6 Significance of the study

This research determined endophytic fungi associated with tree tomato and their effectiveness when applied with *Lantana* leaf extract and Velum® Prime SC 500 (ai = fluopyram 500g/L) in the management of RKNs. Farmers that grow tree tomatoes can use the research's findings to manage RKNs. As an alternative to harmful nematicides, the most potent fungal endophytes (*Colletotrichum nigrum, Aspergillus ustus, Fusarium solani* and *Fusarium oxysporum*) have the potential to be commercialized. To effectively manage RKNs, an integrated nematode management technique which combines *Lantana camara* leaf extract and endophytic fungi can be used. The integrated nematode management strategy that can be a part of the RKN integrated management system has been made possible through the findings of this work. The ability to use an affordable, efficient and environmentally friendly integrated nematode management strategy against RKNs will give farmers the opportunity to increase tree tomato yields and increase revenue generation.

Pathogenicity tests were carried out in the greenhouse to ensure that the *Colletotrichum nigrum* strain used was not pathogenic. This study has shed light on the compatibility of Velum® Prime with Non-pathogenic endophytic *Colletotrichum nigrum* against RKNs on tree tomato. Velum® Prime had no adverse effects on the performance of endophytic fungus. New knowledge on compatibility of Velum® Prime SC 500 (ai = fluopyram 500g/L) with endophytic *Colletotrichum nigrum* offers new strategies in integrated nematode management.

1.7 Conceptual framework

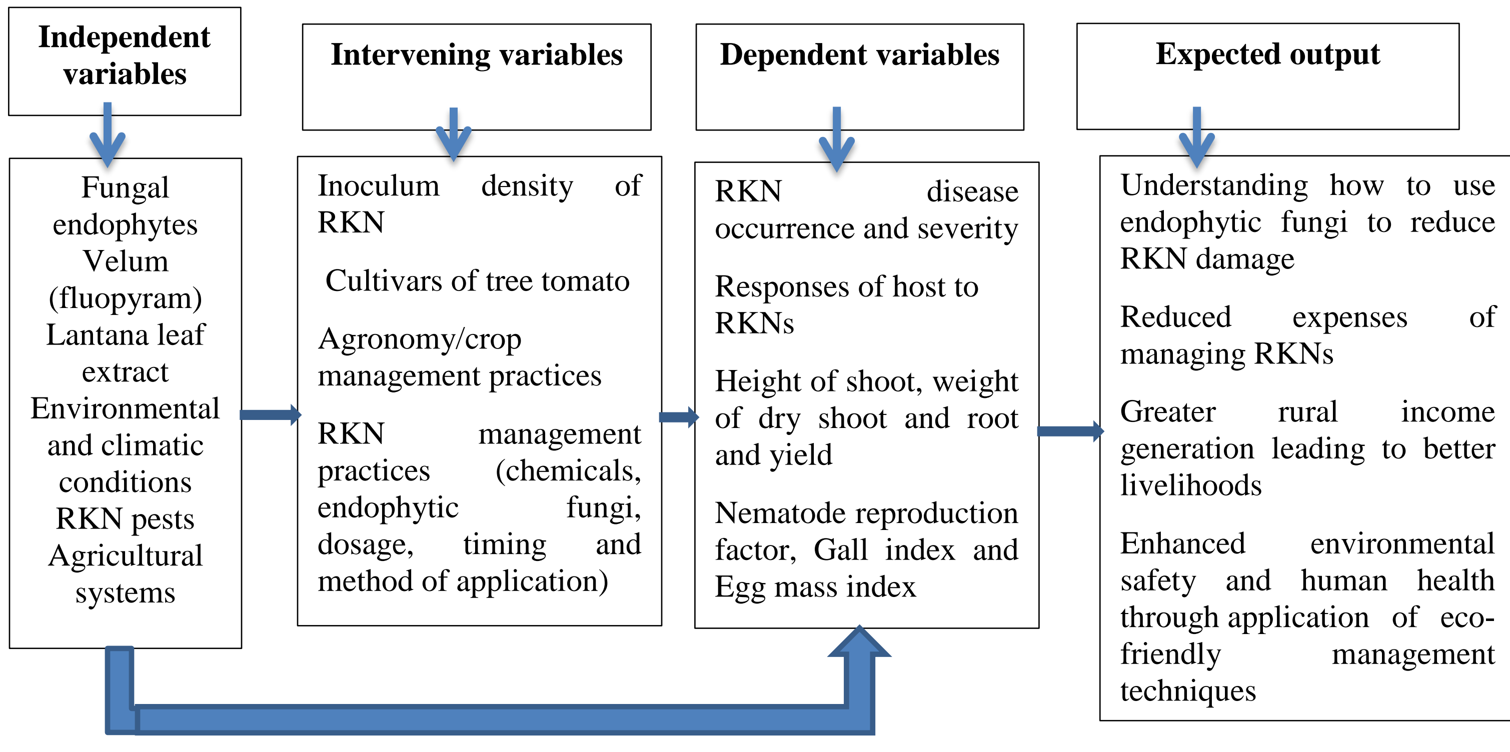


Figure 1:1: Conceptual framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and economic importance of tree tomato

The tree tomato or tamarillo (*Solanum betaceum* Cav.), is a native of Latin America. According to Ramirez *et al*. (2017), the plant, which belongs to Solanaceae family, is cultivated for its fruits, which are consumed raw or processed into juice. Tree tomato is a perennial crop with broad, simple leaves that reach heights of two to three metres. This plant has a woody stem with branches starting at a distance of around 1.5 metres. The plant bears pink-white blooms in clusters of ten to fifty and yields oval-shaped fruits.

The nutritious and health-promoting qualities of tree tomatoes have led to a steady increase in their production in Kenya (AFA, 2021). The production and value of tree tomatoes in Kenya as in 2019 was 17,443 metric tons translating into US$ 7,447,908 (about KES.1 Bllion), respectively, (AFA, 2021) while in 2020 it was 21,776 metric tons translating into US$ 8,461,371.7 (about KES.1.2 Billion), respectively, representing 1.35 % of the overall value of fruits and 0.68 % of the overall value of all horticultural crop produce in Kenya (AFA, 2021). Thus, tree tomato is a lucrative economic venture. Sales of tree tomato fruits generate revenue for farmers in rural areas, enhancing their standard of living (Muriithi *et al*., 2013). Tree tomato seedling propagators sell them for income. In other countries, it is grown as raw materials for industrial processing of syrup and chutney. It is also used in preparation of juice, fruit salads and making of sauce (Rana and Brar, 2019).

2.2 Nutritional importance of tree tomato

Tree tomatoes offer significant nutritional and health benefits because the fruits are loaded with minerals (iron, phosphorus, potassium, and calcium), vitamins (A, B, C, E, and K) and antioxidants (Pedrosa, 2016, Rana and Brar, 2019). The fruit also contains high fiber and low calories in addition to proteins, pectins and carotenes (Rana and Brar, 2019). High levels of vitamin A are important for eyesight improvement, increasing red blood cells (RBCs), and maintenance of skin health. Vitamin C is essential for improved body immunity, healing of wounds and health of the bones. Tree tomato fruits are an important remedy for anemia and respiratory disorders. The proteins present have antimutagenic effect as they reduce oxidative damage and inhibit formation of uric acid in the body. High anthocyanin content shields the body against neurological disorders, diabetes, aging, and some types of cancer. Lycopene in fruits is important in keeping off degenerative diseases; it protects the skin against ultra violet (UV) radiation and improves heart health (Rana and Brar, 2019).

2.3 Production of tree tomato in Kenya

The area under production and production volumes of tree tomato in Kenya was 1,156 Ha, yielding 17,443 metric tons in 2019 (AFA, 2021) while in 2020, it was 1,321 Ha, yielding 21,776 metric tons, respectively (AFA, 2021). Tree tomatoes thrive in tropical and subtropical regions with annual temperatures between 15 and 26 ^{o}C, rainfall ranging from 600 to 4000 mm, and elevations between 1500 and 3000 meters above sea level (masl). The plant thrives in pH 5 - 8.5 rich, free-draining soils (Prohens and Neuz, 2001). It is grown in western Kenya, the Rift Valley, and the counties

surrounding Mount Kenya including Nyandarua, Meru, Embu, Kiambu, Nyeri and Muranga. Goldmine, Rothamer, Red Oratia, Ruby Red, and grafted variants are the principal cultivars in Kenya.

2.4 Tree tomato production constraints in Kenya

The productivity of tree tomato in Kenya is below its potential due to a number of reasons. The main obstacles to production include pests, diseases, and a shortage of certified seedlings and approved seeds. The most dangerous disease is powdery mildew, which is brought on by *Erysiphe* and *Oidium* spp. and can cause the plant to die and lose its leaves. Another issue that many farmers face is shortage of high-quality planting materials (AFA, 2021). In Kenya, there lack of a comprehensive and well-documented scheme for tree tomato seed certification. Seed purity is being challenged by the fact that private nurseries produce the majority of the seed and seedlings. For many small-holder farmers, the average cost of a certified seedling is one US dollar, making it expensive.

Tree tomatoes are also impacted by pests including insects and root-knot nematodes (Muriithi *et al*., 2013; AFA, 2021). A significant challenge to the production of tree tomatoes is posed by root-knot nematodes, which are known to create severe damage and have been extracted from roots of tree tomato in several areas around Kiambu as well as Embu counties (Ramirez *et al*., 2017; Waswa *et al*., 2020). *Meloidogyne javanica*, *M*. h*apla*, and *M*. i*ncognita* severely harm tree tomatoes. When pathogenic bacteria and fungi combine with nematodes to generate disease complexes, there can be a 100% loss (Prohens and Neuz, 2001). Because they consume sap and

spread viruses, aphids (*Myzus persicae*) are another significant pest of tree tomatoes. Up to 75% of crops can be lost due to infestation by green house white fly (*Trialeurodes vaporariorum*), which attacks foliage and fruits. Fruits fall prematurely due to larvae of the tomato worm (*Neoleucinodes elegantalis*) infestation (Prohens and Neuz, 2001). The most significant virus that affects tree tomato is the tamarillo mosaic virus (TaMV). Aphids carry this potent virus, which causes severe fruit browning and chlorotic mottling (Prohens and Neuz, 2001). Other viruses affecting tree tomato includes, tomato aspermy virus (TAV), cucumber mosaic virus (CMV), tamarillo spotted wilt virus (TSWV), arabis mosaic virus (ArMV), tobacco streak virus (TSV), alfafa mosaic virus (ALMV), potato virus Y (PVY) and potato aucuba virus (PAMV) (Prohens and Neuz, 2001).

2.5 Root-knot nematode damage to plants

Root-knot nematodes are sedentary endoparasites of warm tropical and sub-tropical climates around the world and are the top most PPNs that are polyphagous. Root-knot nematodes penetrate roots of plants using a stomato-stylet and move downwards intercellularly to the tip. They enter into the vascular cylinder and eventually reach the zone of differentiation where they induce formation of feeding cells (Escobar *et al*., 2015). Plant nutrients are redirected to the feeding sites and they inject the vascular cells with secretions which cause their redifferentiation into multinucleate hypertrophied cells called giant cells (Bozbuga *et al*., 2018). This result into formation of galls called root-knots. Infected plants exhibit symptoms of galling and root shortening below ground, which obstructs the absorption of minerals and

water. Additionally, plants exhibit indications of wilting, chlorosis of the foliage, thinning, stunting and mortality above ground (Noling, 2000; Jones *et al*., 2013; Agenbag, 2016). Root-knot nematodes damages roots of crops making entry of bacterial, viral and fungal pathogens easier. They also form disease complexes with pathogenic bacteria and fungi resulting into serious damage or even death of plants.

2.6 General life cycle of root-knot nematodes

About 3000 eggs are laid by adult RKN females in a gelatin matrix and as figure 2.1 illustrate, the initial juvenile (J1) molts while in the egg to become the second juvenile stage (J2). After emerging from out of the egg, the RKNs infective stage, known as J2, uses a stylet to pierce roots and moves toward the tip of the root (Figure 2.1). The RKNs have stomato stylet, a structure they use to puncture, inject secretions and feed from the cells. Entering the vascular cylinder from the root tip, it starts permanent feeding sites made up of large cells. The J2s mature into the third juveniles (J3s) after molting and growing in size. The J3 molts into the J4. The J4 then matures into adult females or males which are sexually mature. Male and female RKNs exhibit sexual dimorphism. While the males stay vermiform and exit the roots, the females enlarge and take on a pear-like shape within roots (Jones *et al*., 2013; Agenbag, 2016; Chen *et al*., 2020). The J2s are usually vermiform or worm-like and are mostly found in the soil.

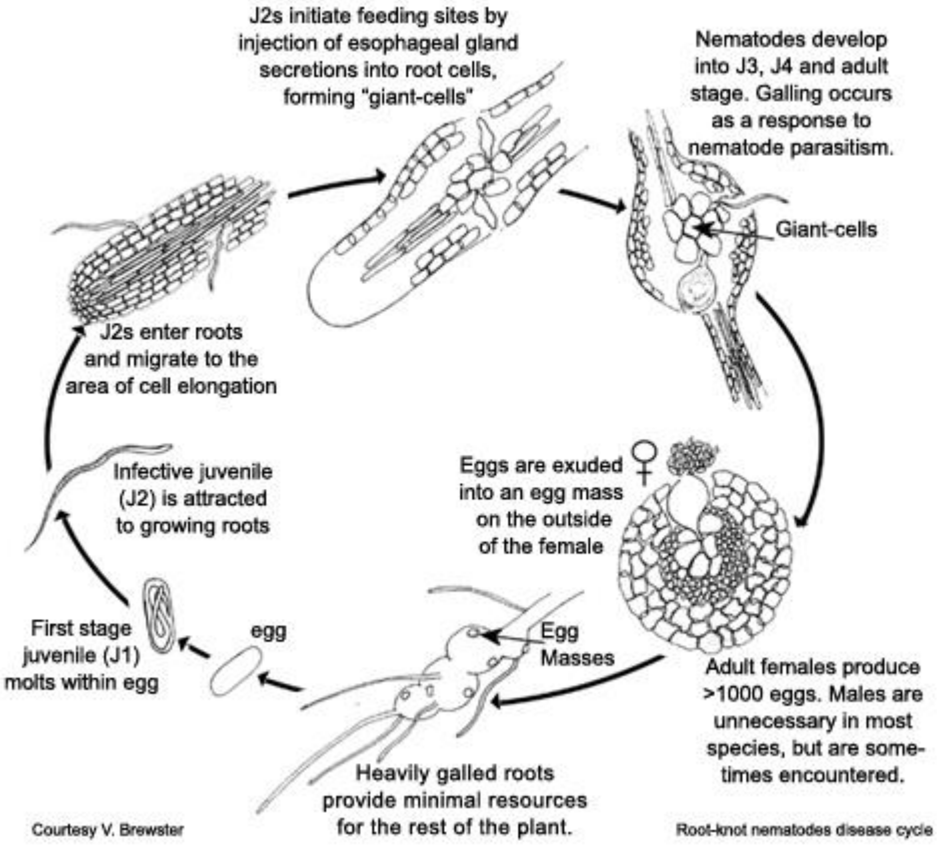


Figure 2:1: Root-knot nematode disease cycle
The second juvenile stage is the RKN infective form that infects the roots of plants inducing formation of galls (Mitkowski and Abawi, 2003)

2.7 Management of root-knot nematodes

Numerous management strategies have been developed in trying to address the RKN issues to reduce losses. Several methods are employed to combat root-knot nematodes, including chemical nematicides, crop rotation, resistant cultivars, organic amendments, physical procedures, local fungal soil isolates and endophytes, and integrated management strategies. On tree tomatoes in Kenya, however, application of an integrated strategy combining endophytic fungus and *Lantana camara* leaf extract against RKNs has not been investigated.

2.7.1 Use of crop rotation strategy

By utilizing crops that are poor-nematode hosts, crop rotation offers the possibility to manage RKNs. However, this approach has proven unreliable because of a number of reasons. The first impediment is that RKNs have vast host ranges (Sikora and Fernandez, 2005). Over 2000 plant species are parasitized by RKNs (Jones *et al*., 2013), and the method's applicability is restricted by the polyphagous nature of RKNs. Secondly, poor hosts like sorghum and sun hemp have been effectively employed against RKNs. However, weeds that act as substitute hosts need to be managed in order for poor host plants to be effective (Noling, 2000). Thus, for efficient crop rotation, an integrated strategy to manage weeds and RKNs should be taken into account (Noling, 2000). Thirdly, this approach may not work effectively especially if the poor host crops are not of high economic value and without ready market (Luna, 1998). This may make them unacceptable by farmers because of low profits and interference in production (Luna, 1998; Kimenju *et al*., 2010). Therefore, identifying high value poor host rotational crops to RKNs is important in the successful implementation of a rotational program. Other crops that have been used as poor-nematode host include sorghum and Sudan grass. Some sorghum varieties produce glycoside in their cells which produces hydrogen cyanide that is highly toxic to nematodes (Kimenju *et al*., 2010) while Sudan grass has been known to produce dhurrin which produces hydrogen cyanide in the soil when it decomposes (Kimenju *et al*., 2010).

2.7.2 Use of resistant cultivars

The most common tree tomato varieties planted in Kenya are grafted on *Solanum mauritianum* rootstock and non-grafted which are Ruby Red, Red Light Oratia, Rothamer, Goldmine and Red Oratia. In research conducted on the prevalence of PPNs and the biological control of RKNs on tree tomato in the Kenyan counties of Kiambu and Embu, it was discovered that Goldmine variety was sensitive to RKNs, while Ruby red, Rothamer, and Red oratia were somewhat resistant (Waswa, 2021). Managing RKNs in an environmentally acceptable way involves using resistant plant varieties. The infectious J2s are prevented from penetrating plant roots, maturing, and reproducing by resistant cultivars. These cultivars might be a part of an integrated pest control strategy (Karuri *et al*., 2017; Sujatha *et al*., 2017). The foregoing approach has presented difficulties because resistance in plants decreases over time when RKN populations and environmental factors change. Furthermore, formation of extremely virulent pathotypes that can overcome plant resistance is caused by considerable diversity in RKN genes (Singh and Khurma, 2007; Maleita, 2011).

2.7.3 Use of soil organic amendments

Use of organic amendments (OAs), such as animal manures and agro-industrial wastes, has shown to decrease the numbers of RKNs (Nchore *et al*., 2016). When added to soil, organic amendments break down and release harmful compounds such as nematotoxic ammonia. They also enhance soil structure, water adsorption, and cation exchange capacity. Antagonistic micro-organisms in the soil develop and multiply by the incorporation of organic matter.

2.7.4 Use of biological control agents

Biological control reduces the damaging effects of the target pathogen and encourages plant growth by utilizing advantageous microorganisms, their by-products, or their genes (De Silva *et al*., 2018). Biological control agents (BCAs) have two ways of interacting with pathogens: directly through antagonistic interactions (antibiosis, competing for nutrients and or space, etc.) or indirectly through the host plant by causing plant resistance, referred to as induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Stirrling, 2018; Xiang *et al*., 2018). Most organisms currently function as biological control agents against PPNs. The bacteria, fungi, viruses, protists, nematode antagonists, and certain invertebrates are among these mircobes (Poveda *et al*., 2020). In integrated management of pest, use of BCAs, physical methods, and cultural practices has proven to be beneficial when combined with low chemical dosages (Poveda *et al*., 2020).

The usage of endophytic fungi against *Meloidogyne* spp. has previously been demonstrated using *Aspergillus* spp., *Trichoderma* spp., *Gliocladum* spp., *Patriella* spp., non-pathogenic *Fusarium* spp., and *Purpureocillium* spp. (Bogner *et al*., 2016; Herrera *et al*., 2016; Larran *et al*., 2016; De Silva *et al*., 2018; Kariuki *et al*., 2021; Musango *et al*., 2021). In both the greenhouse and the field, *Trichoderma asperellum* reduced the soil and root J2s of RKN significantly on tree tomato (Waswa *et al*., 2021). Using local fungal isolates to control RKNs on tomatoes proved effective in a study carried out in Mwea, Kenya (Kariuki *et al*., 2021). Thus, use of local endophytic fungi in biocontrol is a viable approach for managing root-knot nematodes.

However, for fungal BCAs to be as effective against nematodes as possible, a few obstacles must be overcome. Certain plant cultivars and other crops in the farm's rotation have an impact on the effectiveness of certain endophytic fungi; for example, crucifers can decrease endophytic fungi's survivability. Additionally, it is challenging to guarantee that nematode trapping occurs at the same time as RKN and cyst nematode infecting stages. They are also hard to formulate because they don't produce resting spores. Several factors affect the establishment and activity of BCAs for nematodes, including the species of nematode, soil composition, development rates, density, and host plant. The development of the target nematode and the proliferation and sporulation of BCAs are directly impacted by temperature. Moisture rarely inhibits the growth of most fungus, although it does have an impact on the spread of spores. The growth, dissemination, and activity of nematodes are influenced by the texture and structure of the soil (Kerry, 1997).

2.7.4.1 Potential of fungal endophytes as biological control agents

The richness of endophytic fungi offers resources for possible biocontrol agents (Oono *et al.*, 2015; Potshangbam *et al.*, 2017). For a part of their life cycle, fungal endophytes reside in plant tissues asymptomatically and defend plants from disease. They also enhance plant fitness by keeping off abiotic and biotic stresses and improve plant growth and development (Kumar *et al.*, 2023). They can proliferate within or across cells, locally or throughout their host without presenting any signs of disease (Schulz *et al.*, 2015; Lo Presti *et al.*, 2015). Endophytic fungi benefit from internal plant

colonization, which allows the host to protect them from external hazards (Latz *et al*., 2018). Endophytic fungi have demonstrated to be effective against migrating endoparasitic nematodes (*Pratylenchus, Hirschmanniella* and *Radopholus*) and sedentary ones (*Meloidogyne*, *Globodera*, *Sphaeronema*, and *Heterodera* spp.). Certain root endophytes are effective against nematodes at different stages of their life cycle by preventing root penetration, delaying development, and reducing reproduction (Schouten, 2016). Many fungi including *Aspergillus flavus*, *Penicillium chrysogenum* and *Pochonia chlamydosporia* have been used against RKNs (Naz *et al*., 2021). Yao *et al*. (2023) demonstrated the efficacy of endophytic *Acremonnium sclerotigenum* isolated from tomato roots against *Meloidogyne incognita*. Endophytic *Colletotrichum* spp. isolated from tomato plants showed protective and plant promoting abilities (Diaz-Gonzalez *et al*., 2020; Silva Santos *et al*., 2022).

Several biological approaches are shown by endophytic fungi, such as generation of antibiotics, competing for nutrients and space, development of resistance, and mycoparasitism (Bogner *et al*., 2016; Latz *et al*., 2018; Poveda *et al*., 2020) as well as producing metabolites during antibiosis that suppress nematodes (Khan *et al*., 2020; Liu *et al*., 2020). Among these metabolites are alkaloids, phenols, peptides, flavonoids, steroids, quinones, polyketides, terpenoids, and volatile organic compounds (Lugtenberg *et al*., 2016; Latz *et al*., 2018). These metabolites produced by endophytic fungi are toxic to nematodes. Plants harbouring endophytic fungi stimulate an enhanced defense system against pathogen invasion. For instance, *Serendipita indica* has been shown to confer nematode resistance and improve rice plant growth (Nassimi and Taheri, 2017). In mycoparasitism, one fungus parasitizes another, taking nutrients from

it, and finally causing its death (Lecomete *et al*., 2016). Endophytic fungi can be applied in a number of ways, such as adding them to organic fertilizers, drenching in soil, seedling root dips and using them as seed treatments, which reduce the amount of inoculum applied compared to field applications.

2.7.4.2 Factors that influence the efficacy of fungal endophytes in the management of root-knot nematodes

When used in controlled environments, biological control agents show significant effectiveness (Spadro and Gullino, 2005). But when used in the field, a variety of environmental factors such as humidity, wetness, UV radiation, salt, soil texture, temperature change, and soil pH reduce their efficacy (Koll *et al*., 2011). Thus, concentrating on local isolates could reduce this difficulty. Host-dependent BCAs are ideal for biological control because they are shielded from environmental influences that could hinder their growth and effectiveness (Card *et al*., 2016). Other microbial rhizosphere colonizers, weather, and plant host species all have an impact on their success. Research on tree tomato using fungal endophytes in the management of root-knot nematodes has not been investigated in the current areas of study. Thus, the goal of this work was to assess the potential antagonistic effects of locally isolated fungal endophytes against RKNs on tree tomato.

2.7.5 Integrated root-knot nematode management

Root-knot nematodes are one of the main constraints to the production of tree tomato. Considering that there is no single way for eradicating this pest, and because of rising environmental and human health risks, an alternate, environmentally safe strategy of management is required. Combining botanicals with antagonistic fungi in biocontrol have been used for the management of RKNs. *Trichoderma harzianum* when applied along with rape seed, *Lantana*, African marigold, and neem botanicals, the parameters associated with tomato nematode disease decreased (Feyisia *et al*., 2016). Kiriga *et al*. (2018) also reported efficacy of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial farming of pineapple in Kenya. *Purpureocillium lilacinum* strain PL251 combined with Velum® (active ingredient, Fluopyram) significantly reduced RKN populations and disease parameters in tomatoes (Dahlin *et al*., 2019). Antibiotic chemicals synthesized by fungal endophytes are important as biocontrol agents in integrated disease control (Adeleke *et al*., 2022). Endophytic fungi have also been known to secrete plant growth hormones such as indole acetic acid which enhances root development thereby increasing nutrients as well as water accessibility by host plants (Suebrasri *et al*., 2020; Adeleke *et al*., 2022). This research work evaluated the combined utilization of *Lantana camara* L. leaf extract and endophytic fungus as an integrated approach to RKN control.

It has been possible to extract terpinoids, steroids, and flavanoids from *Lantana camara* L. (Verbenaceae). This plant has a variety of bioactive chemicals that have demonstrated nematocidal effectiveness against *M. incognita*. Several compounds have been identified from *L. camara*, including lantadene A, oleanoic acid, lancamarolide,

11α-hydroxy-3-oxours-12-en-28 oic acid, betulinic, lantaninilic acid, and lantadene B among others (Begum *et al*., 2015).

After 72 hours, oleanoic acid was found to be the most effective substance tested , with 82% of J2s dead (Begum *et al*., 2015). Although it was first brought to Africa as an ornamental plant, *Lantana camara* L. has since spread throughout the continent as an invasive weed (Qin *et al*., 2016; Mahloatjie *et al*., 2021). However, allelochemicals present in *Lantana* extracts decreases plant growth at high concentrations but concentrations of 50 % and below stimulates growth of plants (Bordoloi *et al*., 2021; Gebreyohannes *et al*., 2023). Akram *et al*. (2022) demonstrated the efficacy of integrating botanical with biocontrol fungi and Velum® against *Meloidogyne graminicola* on wheat. Integrated use of metam sodium with neem cake and *Purpureocillium lilacinum* significantly reduced RKN disease parameters and increased plant growth parameters in cucumber (Thakur *et al*., 2020). The plant's abundance makes it a good source of raw materials for nematicidal products. The goal of this work was to assess the effectiveness of synthetic nematicide (Velum® Prime), endophytic fungi, and leaf extract from *L. camara* singly and when combined to prevent RKNs on a susceptible variety of tree tomato. The combination under this work has not been tested for managing of nematodes in the areas under study on tree tomato.

2.7.6 Use of chemical control strategy

Crop yield losses resulting from root-knot nematodes are significantly large (Tranier *et al*., 2014). For the most part, chemical management has been utilized to manage soil RKNs and is preferred in intensive cropping systems (Chen *et al*., 2020). Extended use of carbamates and organophosphate nematicides builds nematode

resistance and makes them ineffective in the field (Zhang *et al*., 2017; Chen *et al*., 2020). Environmental contamination is also caused by chemicals (Schouten, 2016). Aldicarb and methyl dibromide contaminate underground water while methyl bromide has an effect on the depletion of atmospheric ozone (Chitwood, 2002; Jones *et al*. 2017). Chemical nematicides are hazardous to un-intended creatures as well as humans. Due to their vast range of effects, chemical nematicides drastically change the flora and fauna of the soil, potentially removing nematode rivals in terms of competition, predation, and parasitism. Methyl bromide for instance, destroys mycorrhizae, which hinders plant growth. Both aldicarb and carbofuran are costly and deadly to birds (Lamovšek *et al*., 2013; Bhattacharjee and Dey, 2014; Onkendi *et al*., 2014; Jones *et al*., 2017). However, use of chemicals with low profile toxicities like systemic non-fumigants alone or in integrated RKN management could be explored. Finding more environmentally acceptable ways to manage RKN to raise the yield and production of tree tomato has become necessary due to market removal of nematicidal chemicals such as carbofuran and methyl bromide (Chitwood, 2002; Ebone *et al*., 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sampling and experimental field sites

Sampling of endophytic fungi and field experiments for seasons I and II were carried out at Kinangop sub-county (-.5546 ^{o} S, 36.5536 ^{o} E) located in Nyandarua County lying at 0.1804^{o}S and 36.5230^{o}E (Figure 3.1). The field season I experiments were carried out between March to June, 2023, at Njabini and season II between August to November 2023, at Engineer (Figure 3.1). Njabini lies at latitude 070053 and longitude 36.66165 and altitude 2584 meters above sea level (masl). Engineer lies at latitude 0606501 and longitude 36.584450, and altitude of 2565 masl. These two areas in Kinangop are located in Upper Highland one (UH1) agro-ecological zone (Jaetzold *et al*., 2010; CIDP, 2023).

The pattern of rainfall in Nyandarua County is bimodal. The long rains fall from March to May with a maximum rainfall of 1700 mm while short rains fall from September to December with a maximum rainfall of 700 mm. The average annual rainfall in Nyandarua County is 1500 mm (CIDP, 2023). The county has temperature range of 12 ^{o}C in July to 25 ^{o}C in December. Night frost occurs before dawn almost every month (CIDP, 2023). The soils are acidic, sand clay loam. The farmers plant different crops such as tree tomato, Irish potatoes, green grams, cabbages, carrots and wheat.

Laboratory and greenhouse experiments were carried out at Kenyatta University main campus (1.1805^{o} S and 369348° E).

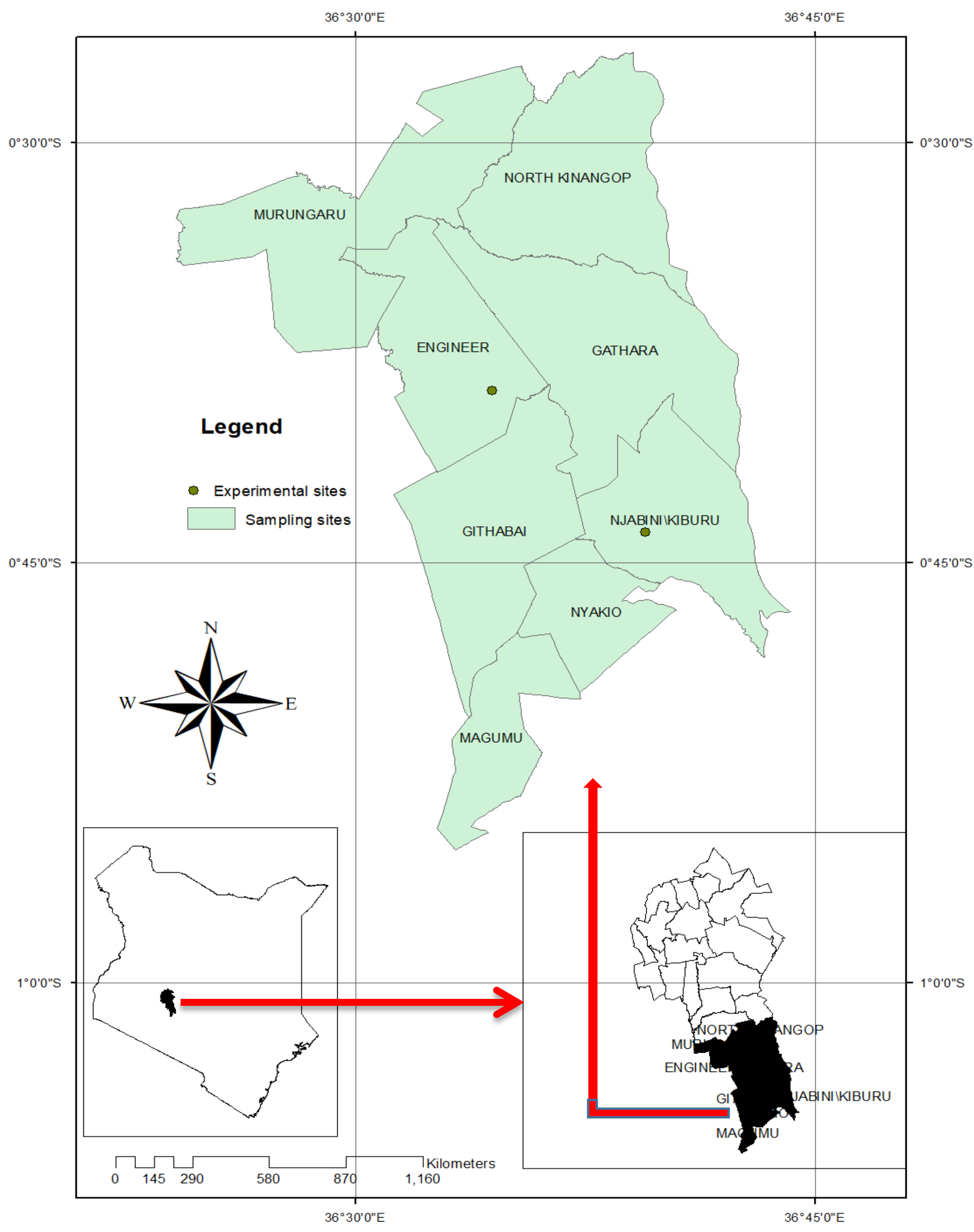


Figure 3:1 Map of Nyandarua county showing Kinangop where sampling and field experiments were conducted.

3.2 General methodologies

3.2.1 Sampling of tree tomato roots for extraction of endophytic fungi

Using the purposive random sampling technique (Suri, 2011), tree tomato roots were sampled from thirty randomly chosen farms in Kinangop, Nyandarua County (Figure 3.1). Three hundred samples were required and ten samples were taken in a zig zag pattern in each farm. The criteria for choosing farms included: farms with more than thirty tree tomato trees, farms that were not on the slope, farms where tree tomatoes were growing alone i.e without intercrops or mixed crops and farms that were 0.5 kilometers apart. Three requirements were taken into consideration while selecting the plants on the farm: they had to be in a physiologically mature stage, devoid of chemical pesticides, and visually healthy. The plants were considered healthy if they were firm strong or vibrant, not wilting, with dark green normal or large leaves without spotting, yellowing or browning. Ten healthy and galled root samples were taken at random in a zigzag fashion from each chosen farm. Plant samples were put inside polythene zip-lock bags, labelled, and stored within a cool box until transportation to the agriculture laboratory at Kenyatta University for further processing.

The proportion of tree tomato plants colonized by endophytic fungi in the farms in Nyandarua County is not known. Therefore, the required sample size was determined using Naing *et al* (2006) formula, where an assumption of 80 % colonization of tree tomato roots by endophytic fungi was adopted using the formula:

$$\mathbf{n} = \frac{\mathbf{pqz^2}}{\mathbf{d^2}}$$

Where $\mathbf{n}$ = minimum sample size required;

$\mathbf{p}$ = proportion of the target plants estimated to be colonized by endophytic fungi;

q = 1-p;

z = precision level (1.96) corresponding to 95 % confidence level;

$\mathbf{d}$ = degrees of freedom (p-value) set at 0.05.

$$\mathbf{n} = \frac{\mathbf{0.8 \times 0.2 \times (1.96)^2}}{\mathbf{0.05^2}} = \mathbf{246}$$

Two hundred and forty six tree tomato plants were required for this study but 300 plants were randomly selected for endophytic fungi isolation from 30 farms located 0.5 kilometers apart. The distribution of the farms was as follows: Magumu = 3; Nyakio = 4; Njabini = 6; Githabai = 4; Engineer = 5; Murungaru = 4 and North Kinangop = 4.

3.2.2 Isolation of endophytic fungi from tree tomato roots

In order to extract fungal endophytes, tree tomato roots that were healthy were gathered from tree tomato farming fields in Nyandarua County. Sterilization of the roots was done as stated by Dababat *et al*. (2008). To get rid of surface epiphytes, the roots were cut into 5-cm-long portions, carefully cleaned with tap water in a sink, and sterilized with 70 % ethyl alcohol for three minutes. The roots were then sterilized using 1.5 % NaOCl (Sodium hypochlorite) for a period of three minutes under a laminar flow hood (BBS-H1100). Sterilized roots were subjected to three rinses with sterilized distilled water; blot dried using sterile blotting papers and cut into 0.5 cm lengths using

sterilized scalpel blades (Dababat *et al*., 2008). Thin roots were sterilized for one minute each in 70 % ethanol and in 1.5 % NaOCl respectively. The 0.5 cm root pieces were equally distributed over potato dextrose agar (PDA) medium, which was autoclave-sterilized for 15 minutes at 121 ^{o}C (39 g of PDA in 1L of sterile distilled water). To ensure that there were no bacterial contaminants in the media, 150 mg/l of streptomycin sulfate was added. Potato dextrose agar plates were sealed and incubated (BJPX-H230JI) at 25 ^{o}C for seven days. The water from the final rinse was plated onto fresh PDA media and incubated at 25 ^{o}C for seven days in order to assess the sterilization integrity. Each isolate was purified on fresh PDA using discs from the leading mycelia margins obtained with a 5 mm flame-sterilized cork borer and placed in an incubator (BJPX-H230JI) for two weeks in order to obtain pure cultures, which were then maintained on fresh PDA media before further use.

3.2.3 Isolation of endophytic fungi from *Meloidogyn*e eggs

Meloidogyne egg masses were picked from galled roots under a dissecting microscope using sterile transfer needles, sterilized with 0.5 % of NaOCl (Hussey and Barker, 1973) for 4 minutes to dissolve the egg masses. The egss were rinsed on 20µm sieve and rinsed with sterile distilled water for 5 minutes and placed aseptically on PDA amended using streptomycin (150 mg/l). The media plates were wrapped with parafilm and incubated (BJPX-H230JI) for seven days at 25 ^{o}C. To obtain pure cultures, freshly prepared PDA was subjected to sub-culturing. For 14 days, observations were made every three days. Prior to use, the pure cultures were maintained on fresh PDA media.

3.2.4 Preparation of fungal inoculum

The fungal isolate that exhibited the greatest nematicidal activity on J2s *in vitro* (Section 3.3.1) was selected, purified and kept at 25 ^{o}C for 14 days on PDA media (Section 3.2.2). For mortality of J2s tests, spore suspensions were made by flooding the surface of seven day old pure fungal cultures with 10 ml of sterilized distilled water amended with 1 % tween 20. Then, a sterile microscope slide was used to dislodge the fungal spores by gentle scrapping of the mycelia on the surface of PDA. A hemocytometer was used to measure the spore densities under the microscope after the contents had been filtered through three layers of muslin cloth (Niranja *et al*., 2009). Water that had been distilled and sterilized was added to adjust the spore density to 1×10^6 spores per ml for use.

Using dried sorghum grains, the spores of the most potent endophytic fungal isolate (assessed against J2s *in vitro*) were mass-multiplied in accordance with Cumagun and Moosavi's (2015) protocol for greenhouse and field experiments. Four 5 mm discs from five-day-old cultures per 200g were used to inoculate the grains after they had been soaked in water, autoclaved, and then placed in polythene bags for a 14-day incubation period. After being air-dried, the grains were ground into powder and sieved using a 50µm sieve. The spore powder was then carefully combined with sterile talc powder as the carrier material in a 1:2 ratio and 5 g/kg of carboxymethyl cellulose as sticking agent (Sing *et al*., 2016). One gram of dried product was suspended, and the fungal isolate's desired spore density was determined by serially diluting it. A calibrated hemocytometer was used to quantify the fungal isolate's precise spore density (1×10^6 spores/ml) for use in further investigations.

3.2.5 Preparation of root-knot nematode inoculum

For three months in the greenhouse, the second stage juveniles of RKN were raised on the susceptible tomato cultivar, Cal-J. To produce nematode inoculum, galled roots from tree tomato plants in Nyandarua County were collected. To obtain pure cultures for the studies, a needle was used to remove one egg mass from a single female. Four-weeks-old transplanted susceptible tomato plants were placed in pots (12 cm diameter) containing sterile 2 sand to 1 soil mixture with *Meloidogyne* egg mass placed at the roots. Pots were maintained in the greenhouse and the plants were removed after three months, their galled roots cleaned, and cut into one-centimeter pieces, macerated in 1.5 % NaOCl solution in a blender and the suspension passed through 500 µm, 106 µm and 20 µm sieves into a beaker (Hooper *et al*., 2005). The ensuing suspension containing RKN eggs was incubated on plates lined with a serviette in darkness for a period of 14 days. The J2s (freshly hatched) were recovered from the fourth day and thereafter every two days (Coyne *et al*., 2007). Using a grid-49 nematode counting dish, the number of newly hatched J2s per millilitre was counted under a microscope at ×40 (Hussey and Barker, 1973). The suspension of nematodes was diluted with sterile distilled water to 50 J2s/ml for use in the laboratory and to 2000 J2s/ml for use in the greenhouse. The freshly prepared nematode suspensions were used immediately.

3.2.6 Nursery establishment

A certified nursery (Kenya Agriculture, Livestock and Research Organization at Horticulture Research Institute) provided the susceptible cultivar (Goldmine) of tree tomato seeds, which were then immersed in sterilized distilled water for a day. After soaking, the seeds were placed in sterile petri plates, lined with moist tissue paper and wrapped with Parafilm. The petri plates were covered with aluminium foil and kept on the benches in the greenhouse for 12 to 15 days. Following germination, the seeds were placed in sterile media-filled germination trays and maintained for four weeks before being transplanted into pots.

3.2.7 Preparation of growth media for greenhouse experiments

A 2:1 proportion of sand to soil was thoroughly mixed and heat-sterilized to 100 degrees Celsius for 48 hours. To prevent contamination, 2 kgs of the sterilized media were placed in thoroughly cleaned plastic pots (12-cm-diameter) that had been sterilized with 1.5 % of NaOCl.

3.2.8 Seedbed preparation for field experiments

The land, with RKN infestation (10^4) occurring naturally was demarcated, ploughed, and prepared into plots of 3 m by 4 m. On every plot, the initial nematode populations (Pi) were established. To do this, 100 cm^3-soil samples were randomly taken at ten locations throughout each plot in a zig-zag pattern. The samples were composited and 200 cm^3 sub-sample was used to extract nematodes for each plot.

Before planting, this process was carried out for every other plot. Twelve plants were planted in each plot, with spacing of 80 cm within rows and 100 cm inter rows.

3.2.9 Evaluation of root-knot nematode disease parameters

Using a modified Baermann method, the J2s of RKN were recovered from 200 cm^3 of composited sub-sample of soil or 5 g of composited sub-sample of roots (Hooper *et al*., 2005; Coyne *et al*., 2007). A five-gram of roots composited sub-sample was cleaned, cut into one-centimeter pieces, and macerated for one minute and the method described above was used to extract nematodes. In order to extract the nematodes, the set-ups were incubated for 48 hours. The suspension of nematodes was then transferred onto a 20µm sieve, gently backwashed, collected into a beaker, and then concentrated using a test sieve to 10 ml. Subsequently, a 2 millilitre suspension of nematodes was pipetted into a dish for counting nematodes. Under a dissecting microscope, the nematodes were counted at ×40 and tallied from the initial concentrated suspension for every aliquot. Two hundred cubic centimetres of dry soil or 5 g of dry roots were used to express the nematodes.

On a scale of 0 to 5, the indices of galling and egg masses were evaluated (Quesenberry *et al*., 1989) as shown in Table 3.1 below. Individual root systems were washed and galling/egg mass indices were scored.

Table 3.1: Scoring scale for root-knot nematode galling and egg mass indices

Score	Description
0	No galls or No egg masses
1	1-2 galls or egg masses
2	3-10 galls or egg masses
3	11-30 galls or egg masses
4	31-100 galls or egg masses
5	>100 galls or egg masses

The galls and egg masses were scored per root system.

Root systems were stained with phloxin-B using Holbrook *et al*. (1983) protocol in order to observe RKN females and egg masses. After soaking in phloxine-B for 20 minutes, stained roots were washed using tap water to get rid of any leftover stain before being examined under a dissecting microscope (Daykin and Hussey, 1985).

After termination of the experiments, final nematode populations (Pf) were evaluated on each plot. To execute this, 100 cm^3 soil samples were randomly taken at ten locations at root rhizosphere of each plant on each plot. The samples were composited and 200 cm^3 sub-sample was used to extract nematodes for each plot using modified Baermann method. The ratios of final nematode populations (Pf) to initial nematode populations (Pi) were used to calculate the nematode reproduction factor (Rf).

3.2.10 Evaluation of plant growth parameters

A centimeter graduated ruler was used in the measurement of plant shoot height from the newest apical shoots and the soil baseline at the conclusion of the experiment. At closure of the experiment (90 days after transplanting), the dry weights of root and shoot were measured in grams. Plants were gently removed from soil, and shoots and roots were separated by cutting at the base. To remove adherent soil, roots were washed in flowing water under the tap and dried with blotting paper. The roots and shoots were placed in paper bags with clear labels. To get consistent mass, the samples were oven-dried for three days at 60 °C.

3.3 Specific methodologies

3.3.1 Efficacy of endophytic fungi on mortality of RKN J2s *in-vitro*

Five millilitre sterile falcon tubes were filled with one millilitre of the suspension of the fungi containing 1 x 10^6 spores of each isolate. Fifty *Meloidogyne* J2s in 1ml were added into the tubes and kept for seventy two hours at room temperature of 23 ^{o}C (Shawky *et al*. (2010). A hemocytometer was used under a microscope to measure the spore density. One millilitre of sterilized distilled water with J2s made up the control treatment. The suspension of nematodes was passed through a tiny (11µm) sieve that was set over a 250 ml glass beaker filled with water and kept in the dark for an hour in order to separate the active and healthy J2s from the docile ones. The active and healthy J2s settled at the bottom of the beaker after swimming through the sieve. The water above was pipetted off to concentrate the J2s, which were then used

immediately. Each treatment had four replicas set up in completely randomized design (CRD) in the laboratory. At the end of 24, 48, and 72 hours, the number of dead J2s was counted in a counting dish under a microscope. Nematode J2s were deemed dead if they were rigid and straight and showed no reaction when a mounting needle was used to probe the tail (Abbasi *et al.*, 2008). The experiment was repeated once. At the closure of the experiments, the J2s of RKN were transferred into sterile distilled water and left for 72 hours to check for any recovery. The J2s did not recover from mortality. Data of dead J2s was converted into percentage mortality using the formula by Abbot. (1925) before statistical analysis:

$$\textbf{Percent mortality of J2s} = \frac{\textbf{Dead J2s}}{\textbf{Total J2s}} \times \textbf{100}\ (\text{Abbot}, 1925)$$

3.3.2 Identification of endophytic fungi causing mortality of RKN J2s

3.3.2.1 Morphological identification of endophytic fungi

Endophytic fungi that showed 50 % and above mortality of J2s from the *in vitro* experiments were identified microscopically, macroscopically and by DNA sequencing. Mycelia discs with diameter of five millimetres, derived from pure cultures of distinct isolates, were cultured onto fresh PDA media in petri plates and maintained for seven days at 25 ^{o}C. The color of the colonies and morphology was determined macroscopically (Appendix 6). Microscopically, features at a magnification of ×1000 were identified. On a slide, mycelia and spores from colonies that were actively growing were stained in a drop of lactophenol cotton blue. The conidia images (Plate 4.2) were taken by Euromex VC 3036 camera mounted on Euromec microscope

connected to a monitor. Using fungal identification keys in addition to the kind of mycelia, conidia morphology, and the presence or absence of macroconidia, microconidia, and chlamydospores, isolates were identified down to the species level (Watanabe, 2010).

3.3.2.2 Molecular identification

Each fungus was inoculated onto PDA culture plates to produce mycelia. Five-day old plate cultures were used to harvest mycelia, which were then frozen, lyophilized for a day, and ground. The DNA was extracted from 20 mg of pure lyophilized mycelia using the Quick-DNA^{TM} Fungal minirep kit (Zymo Research, Inqaba Biotech, South Africa) as per the manufacturer's instructions.

3.3.2.3 Polymerase chain reaction

The PCR mixture had a final reaction volume of 20 µl (consisting of 10 µl of NEB OneTag 2 × Master Mix with standard buffer, 1 µl of genomic DNA, 1 µl forward primer, 1 µl reverse primer, and 7 µl of nuclease free water). Internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer sets (White *et al*., 1990). The amplification was carried out in a gradient thermo cycler (Applied Biosystems) with the following conditions: initial denaturation at 94 ^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at 50 ^{o}C for 30 seconds and extension at 68 ^{o}C for 1 minute. The final extension was

carried out at 68 ^oC for 10 minutes. To determine the success of PCR amplification, gel electrophoresis was carried out to confirm the products.

3.3.2.4 Gel electrophoresis

On 1 % (w/v) agarose (1 g agarose powder into 100 mg of 1 × TBE buffer), the PCR amplicons were electrophoresed, stained with 2µl of SYBR Green and 2µl of Ethidium bromide dye. The mixture was poured into a casting tray containing a comb to solidify. A 2µl volume of each product was loaded in each well. A voltage of 100V was passed to the gel and ran for 30 minutes to enable movement of PCR products while visualization of the gel was done under UV light whereby appearance of clear bands signified the success of PCR amplification.

The products of PCR were purified using ExoSAP protocol. The purified products of PCR were sent to Inqaba African Genomic platform in South Africa for Sanger sequencing.

3.3.2.5 Bioinformatic analysis

The ambiguous sections on the sequences were trimmed and deleted from the chromatogram in Chromas version 2.6.6. The consensus sequences were generated for each endophytic fungus generating the reverse complement of the forward sequences in Biological sequence alignment Editor (BioEdit). The consensus sequences were BLAST (Basic Local Alignment Search Tool) against NCBI (National Centre for Biotechnology

Information) nucleotide databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using BlastN tool. The phylogenetic analysis and ITS consensus sequences obtained for each fungal isolate and those retrieved from GeneBank's NCBI database were aligned in clustalW with gamma parameter of 1.00, an opening gap penalty of 15.00 and extension of 6.66 in both pairwise and multiple alignments using MEGA 11 (Molecular Evolutionary Genetic Analysis) package (Tamura *et al*, 2021) to determine their evolutionary relationships. The Phylogenetic tree was built in MEGA 11 using Neighbor-joining method (Saitou and Nei *et al*, 1987) with 1000 bootstrap replications (Felsenstein, 1985). The evolutionary distances of the number of base substitutions were computed using Tamura method (Tamura, 1992).

3.3.3 Compatibility of Velum® Prime with selected endophytic fungus

3.3.3.1 *In vitro* experiment

Velum® Prime SC (suspension concentrate) 500 (ai = fluopyram 500g/L) was procured from Elgon Kenya limited (BAYER). Before solidification, 1 ml of Velum® Prime suspension (1 ml Velum/L) was dispensed into a 1 L conical flask containing 1L sterilized molten PDA media and gently agitated in order to mix evenly. After pouring the mixture into 90 mm petri dishes, it was left to cool and solidify. Commercial *Trichoderma asperellum* (TRC900)® was obtained from Real IPM in Kenya. Using a sterile 5 mm cork borer, the discs from the actively growing edge of the endophytic *Colletotrichum nigrum* or *Trichoderma asperellum* fungal colonies were removed and inoculated onto PDA media modified with Velum® Prime. Control plates contained

un-amended sterile PDA media. The PDA plates were sealed with parafilm and incubated for seven days at 25 ^{o}C. The treatments were replicated four times each and arranged in CRD. The treatments included:

i) Plates amended with Velum® Prime + endophytic *Colletotrichum nigrum* isolate

ii) Plates amended with Velum® Prime + Commercial *Trichoderma asperellum*

iii) Un-amended plates treated with *C. nigrum* (control)

iv) Un-amended plates treated with *T. asperellum* (control)

From the underside of each petri dish, the colony diameter (radial growth) was measured. The percentage inhibition of growth over the control was calculated using the formula:

$\mathbf{I} = \frac{\mathbf{C-T}}{\mathbf{C}} \times \mathbf{100}$ (Behdani $et\ al.$, 2012)

Where **I** = percent inhibition, **C** = colony diameter in control plate, and **T** = colony diameter in treated plate. The experiment was repeated once for accuracy.

3.3.3.2 Greenhouse experiment

In the greenhouse, sterile plastic pots (12 cm diameter) were filled with unamended sterile soil media (2 sand: 1 soil). Forty millilitres (40 ml) of 1 ml/L Velum® Prime (ai = fluopyram SC 500g/L) nematicide was poured into the planting holes in the soil in pots prior to transplanting. One tree tomato seedling that was four weeks old was transplanted into each pot. Before being transplanted, seedlings were

treated with 1 $\times$ 10^6 spores/ml of either commercial or endophytic fungal isolate as seedling root dip for 2 hours. Pots were inoculated with 2000 J2s of RKN each one week after transplanting. Pots inoculated with J2s of RKN only served as positive control while un-treated pots without nematode J2s served as negative control. The treatments had six replicates each arranged in CRD. The treatments were:

i) *Colletotrichum nigrum* + Velum® Prime + RKN J2s

ii) *C. nigrum* + Velum® Prime – RKN J2s

iii) *Trichoderma asperellum* + Velum® Prime + RKN J2s

iv) *T. asperellum* + Velum® Prime - RKN J2s

v) *C. nigrum* + RKN J2s

vi) *C. nigrum* - RKN J2s

vii) *T. asperellum* + RKN J2s

viii) *T. asperellum* - RKN J2s

ix) Velum® Prime + RKN J2s

x) Velum® Prime - RKN J2s

xi) RKN J2s alone

xii) Untreated and un-inoculated

Ninety days after transplanting, the experiments were concluded. Plant growth measurements and severity of RKN disease were assessed at the end of the experiments, as stated in sections 3.2.9 and 3.2.10, respectively. One repetition of the experiment was conducted.

3.3.4 Efficacy of endophytic fungus combined with *Lantana camara* leaf extract and Velum® Prime against root-knot nematodes

In vitro tests were set up in a lab and repeated once, whereas the greenhouse and field trials were set up over the period of two seasons. Mature *Lantana* leaves were picked from the hedges of Kenyatta University agricultural farm, in their natural habitat. After being shade-dried for 3 weeks and processed through an electric grinder to a fine powder, 20 grams of the leaf powder were soaked for 24 hours in 100 ml of sterilized distilled water in a 500 ml conical flask. The filtrate was passed through two folds of muslin clothes. For *in vitro* investigations, the suspensions were filtered through Whatmann No. 1 filter paper after 24 hours and the ensuing filtrate centrifuged at 2400 revolutions per minute (rpm) for 10 minutes. The extract was treated as a 'standard solution' or the base (100 percent concentration). Using sterile distilled water, suspensions at concentrations of 25, 50, 75, and 100 percent (Taye *et al*., 2012) were made from the standard solution. The extract was used immediately after constitution. For greenhouse experiments, the 50 % concentration was added to pots after nematode inoculation, and for field tests, it was drenched around the roots during transplanting. The 50 % concentration was chosen because a concentration above 50 % is known to inhibit plant growth parameters due to phytotoxicity (Bordoloi *et al*., 2021).

3.3.4.1 Efficacy of *Lantana camara* leaf extract and endophytic fungi on J2s of RKN *in vitro*

One millilitre of 0, 25, 50, 75, 100 percent concentrations of *Lantana camara* L. extract were pipetted into sterile Eppendorf tubes and 1 ml of juvenile suspension containing 50 J2s pipetted into each.

In another set, 1 ml containing 50 J2s was pipetted into sterile Eppendorf tubes having 1 ml of varying concentration of *Lantana* leaf extract and 1 ml of 1×10^6 of the endophytic fungal isolate. The control treatment had 1 ml of sterilized distilled water in Eppendorf tubes containing 1ml of 50 J2s. Four replicates of each experiment were set up in CRD in the laboratory. The number of dead and active J2s in each treatment was counted after 24, 48, and 72 hours. The juveniles (J2s) were deemed dead if they were rigid and straight and showed no reaction when a mounted needle was used to probe the tail (Abbasi et al., 2008). The experiments were repeated once. At the closure of the experiments, the RKN J2s that were dead were transferred into sterilized distilled water and left for a period of 72 hours to check for any recovery. The dead J2s did not recover from mortality. Data of dead J2s was converted into percentage mortality before statistical analysis using the formula by Abbott. (1925) as follows:

$$\textbf{Percent mortality of J2s} = \frac{\textbf{Dead J2s}}{\textbf{Total J2s}} \times \textbf{100}$$

The treatments of using *L. camara* leaf extract alone were as follows:

i) 25 % *Lantana* leaf extract + 50 RKN J2s

ii) 50 % *Lantana* leaf extract + 50 RKN J2s

iii) 75 % *Lantana* leaf extract + 50 RKN J2s

iv) 100 % *Lantana* leaf extract + 50 RKN J2s

v) Sterile distilled water + 50 RKN J2s

The treatments of combining *L. camara* leaf extract with *C. nigrum* were as follows:

i) 25 % *Lantana* leaves extract + *C. nigrum* (1×10^6spores/ml) + 50 RKN J2s

ii) 50 % *Lantana* leaves extract + *C. nigrum* (1×10^6spores/ml) + RKN J2s

iii) 75 % *Lantana* leaves extract + *C. nigrum* (1×10^6spores/ml) + RKN J2s

iv) 100 % *Lantana* leaves extract + *C. nigrum* (1×10^6spores/ml) + 50 RKN J2s

v) *C. nigrum* (1×10^6spores/ml) + 50 RKN J2s

vi) Sterile distilled water + 50 RKN J2s

3.3.4.2 Greenhouse experiment

In the greenhouse on Goldmine tree tomato, the effectiveness of *Colletotrichum nigrum* isolate selected based on exhibiting the highest mortality of J2s (section 3.3.1), *L. camara* leaf extract, Velum® Prime SC 500 (ai = fluopyram 500g/L), and commercialized *Trichoderma asperellum* against J2s of RKN were evaluated. Sterilized soil media (2 sand: 1 soil) was put into 12 cm diameter pots. Four-week old Goldmine tree tomato seedlings were put in a suspension of fungal endophytes by their roots (before transplanting, the fungal isolates were applied as root dips for seedlings, containing 1×10^6 spores/ml, for two hours). One week after transplanting, pots were inoculated with 2000 J2s of RKN by making three 1cm holes around the roots. A week from nematode inoculation (two weeks after transplanting), 250 ml of 50 % concentration of the botanical leaf extract of *L. camara* was administered as soil drench around roots in pots. Forty millilitres of Velum® Prime (ai = fluopyram 500g/L) was

applied around the roots in pots together with leaf extract of *L. camara*. Commercial *Trichoderma asperellum* was applied at 40 ml containing 1×10^6 spores/ml in each pot. Control pots received 40 ml drench of sterilized distilled water. Every treatment had four replicas in the greenhouse in CRD and kept at 26 ^{o}C. Two control treatments were set up: positive controls pots were infected with nematodes only and left untreated, while negative controls pots were not inoculated and left untreated. Ninety days after transplanting, the greenhouse tests were terminated. The parameters of nematode disease and growth of plants were determined at the end of research, as outlined in sections 3.2.9 and 3.2.10, respectively. The greenhouse treatments were as follows:

i) *Colletotrichum nigrum* + RKN J2s

ii) *C. nigrum* - RKN J2s

iii) *Trichoderma asperellum* + RKN J2s

iv) *T. asperellum* - RKN J2s

v) Velum ® Prime + RKN J2s

vi) Velum® Prime - RKN J2s

vii) *Lantana* leaf extract (LE) + RKN J2s

viii) *Lantana* leaf extract - RKN J2s

ix) *C. nigrum* + LE + Velum® Prime + RKN J2s

x) *C. nigrum* + LE + Velum® Prime - RKN J2s

xi) *T. asperellum* + LE + Velum® Prime + RKN J2s

xii) *T. asperellum* + LE + Velum® Prime - RKN J2s

xiii) *C. nigrum* + LE + RKN J2s

xiv) *C. nigrum* + LE - RKN J2s

xv) *T. asperellum* + LE + RKN J2s

xvi) *T. asperellum* + LE - RKN J2s

xvii) RKN J2s alone (positive control)

xviii) Untreated and un-inoculated (negative control)

3.3.4.3 Field experiments

Experiments were set up on 3 m by 4 m plots naturally infested with RKNs (10^4) at Kinangop with four replicas of treatments using Randomized Complete Block Design (RCBD). Before transplanting, the fungal isolates (1×10^6 spores/ml) were applied as root dips for seedlings for two hours. A 250 ml solution containing 50 % *Lantana* leaf extract suspension was drenched around the seedlings' roots. Fifty percent concentration of *L. camara* was chosen because research has shown that concentrations above 50% reduce plant growth. Velum® Prime (ai = fluopyram 500g/L) was applied as soil drench at 40 ml during transplanting. Commercial *Trichoderma asperellum* was applied at 40 ml containing 1×10^6 spores/ml in each planting hole. Plots without treatments served as control. Ninety days away from transplanting, the field tests were ended and the findings regarding nematode disease and plant development determined as detailed in sections 3.2.9 and 3.2.10, respectively. SAS version 9.2 software was used to do an Analysis of Variance on the data involving disease variables and plant development that were recorded both in the greenhouse and field. The field treatments were as follows:

i) *Colletotrichum nigrum* + RKN J2s

ii) *Trichoderma asperellum* + RKN J2s

iii) *Lantana* leaf extract (LE) + RKN J2s

iv) Velum® Prime + RKN J2s

v) *C. nigrum* + LE + Velum® Prime + RKN J2s

vi) *T. asperellum* + LE + Velum® Prime + RKN J2s

vii) *C. nigrum* + LE + RKN J2s

viii) *T. asperellum* + LE + RKN J2s

ix) RKN J2s alone (un-treated plots-positive control)

3.4 Data analysis

The data organization was done in excel sheets and subjected to analysis of variance (ANOVA) using SAS (version 9.2) computer software. To distinguish between means that were significant, the Tukey's Honestly Significant Differences test (HSD, where $P \leq 0.05$) was employed. The obtained data was checked for normality before ANOVA using Shapiro-Wilki test. Relationship between plant disease variables was assessed using regression and correlation analyses.

CHAPTER FOUR: RESULTS

4.1 Efficacy of endophytic fungal isolates on mortality of RKN J2s

All the 44 fungal isolates were tested for efficacy against RKN J2s based on mortality *in-vitro*. Out of the 44 isolates, 20 showed more than fifty percent mortality of J2s in both *in-vitro* tests (Tables 4.1 and 4.2; Plate 4.1 A-E). *Colletotrichum nigrum* caused highest mortality of J2s at 87 % followed by *Aspergillu*s *ustus* and *Fusarium solani* (63.5 % each) and *F. oxysporum* (63.0 %) while the least was *Colletotrichum coccodes*, (50.5 %) in test I (Table 4.1; Plate 4.1 E). There were statistical differences ($P \leq 0.05$) in the efficacy of fungal isolates on mortality of J2s (Tables 4.1 and 4.2). In the *in-vitro* test II, *C. nigrum* had the highest mortality of J2s of 85 % followed by *A.ustus* (66 %), *F. solani* (65 %) and *F. oxysporum* (63 %) while the least was *Rhizoctonia solani* (51.5 %) after 72 hours (Table 4.2).

Table 4.1: Efficacy of endophytic fungal isolates on mortality (%) of RKN J2s in *in-vitro* test I

	% Mortality of RKN J2s treated with endophytic fungi isolates			
Code	**Endophytic fungal isolate**	**24 Hours**	**48 Hours**	**72 Hours**
N2	*Colletotrichum nigrum*	33.00 ± 3.14a	51.50 ± 3.50a	87.00 ± 2.08a
N5	*Fusarium oxysporum* f. sp. *ciceris*	23.00 ± 1.29abd	34.50 ± 1.71bcdef	63.00 ± 1.91bc
N10	*Aspergillus oerlinghausensis*	11.00 ±1.30e	19.50 ± 3.29ij	55.00 ± 1.73bcd
N11	*Fusarium oxysporum*	15.00 ± 0.58de	30.00 ± 1.83cdefghi	52.00 ± 0.96d
N14	*Microsphaeropsis arundinis*	17.00 ± 1.29bde	24.50 ± 1.50fghij	51.50 ± 1.71d
N17	*Aspergillus* spp.	10.5 ± 1.50e	39.50 ± 1.89bc	56.0 ± 0.82bcd
N18	*Aspergillus ustus*	27.00 ± 4.04ab	31.00 ± 3.79cdefgh	63.50 ± 1.71b
N20	*Penicillium onobense*	26.00 ± 2.58ab	35.50 ± 1.71bcdefg	53.00 ± 1.91d
N24	*Penicillium janthinellum*	14.50 ± 1.71e	18.50 ± 1.50j	52.00 ± 1.83d
N26	*Fusarium solani*	24.00 ± 1.41abd	45.50 ± 2.22ab	63.50 ± 1.71b
N27	*Rhizoctonia solani*	25.30 ± 3.64ab	39.00 ± 1.29bc	51.50 ± 1.73d
N28	*Colletotrichum coccodes*	18.50 ± 0.96bde	23.5 ± 1.71ghij	50.50 ± 1.26d
N29	*Didymella bellidis*	23.50 ± 1.89 abd	27.50 ± 1.89defghij	56.00 ± 0.82bcd
N31	*Periconia* spp.	11.50 ± 1.71e	22.00 ± 1.41hij	55.00 ± 2.65bcd
N33	*Didymella bellidis*	24.00 ± 1.83abd	34.50 ± 0.96bcdefg	57.00 ± 1.29bcd
N34	*Aspergillus* spp.	8.50 ± 0.96ef	27.50 ± 2.87defghij	54.50 ± c1.71d
N36	*Fusarium fujikuroi*	27.00 ± 2.08ab	37.00 ± 3.11bcd	56.50 ± 2.63bcd
N41	*Colletotrichum coccodes*	15.00 ± 1.29de	25.00 ± 3.11fghij	54.00 ± 1.41d
N43	*Aspergillus fumigatus*	17.00 ± 1.08 bde	33.80 ± 0.85cdefg	54.00 ± 0.85d
N44	*Purpureocillium lilacinum*	17.50 ± 0.65bde	36.30 ± 1.11bcde	54.75 ± 1.08cd
	Control	0.0 ± 00f	0. 25 ± 0.25k	0.50 ± 0.29e
	P-Value	<.0001	<.0001	<.0001

Means with different letter(s) in a column are statistically different according to Tukey's Honestly significant difference (HSD) test at P≤0.05. Every value (n = 4) is the average of four replicates.

Table 4.2: Efficacy of endophytic fungal isolates on mortality (%) of RKN J2s in *in-vitro* test II

		% Mortality of RKN J2s treated with endophytic fungi isolates		
Code	**Endophytic fungal isolate**	**24 Hours**	**48 Hours**	**72 Hours**
N2	*Colletotrichum nigrum*	32.50 ± 2.50a	52.00 ± 0.82a	85.00 ± 2.38a
N5	*Fusarium oxysporum* f. sp. *ciceris*	21.00 ± 1.29bcdefg	38.50 ± 01.71bcd	63.00 ± 1.29bcd
N10	*Aspergillus oerlinghausensis*	12.00 ± 1.41hij	17.00 ± 1.29j	55.50 ± 0.96de
N11	*Fusarium oxysporum*	18.50 ± 1.71bcdefgh	24.50 ± 1.71fghij	53.00 ± 1.29e
N14	*Microsphaeropsis arundinis*	17.50 ± 1.26bcdefghi	21.50 ± 0.96ij	54.00 ± 1.63e
N17	*Aspergillus* spp.	16.00 ± 183efghi	38.00 ± 1.83bcd	57.00 ± 1.29e
N18	*Aspergillus ustus*	27.50 ± 2.99abc	34.50 ± 3.30bcdef	66.00 ± 2.16b
N20	*Penicillium onobense*	24.00 ± 2.45bcde	37.50 ± 1.71bcd	54.00 ± 2.16e
N24	*Penicillium janthinellum*	13.50 ± 1.50ghij	18.00 ± 1.41j	54.00 ± 1.63
N26	*Fusarium solani*	19.50 ± 0.96cdefgh	42.50 ± 2.75ab	65.00 ± 1.73bc
N27	*Rhizoctonia solani*	29.00 ± 1.91ab	42.00 ± 1.41abc	51.50 ± 1.50e
N28	*Colletotrichum coccodes*	15.00 ± 0.96fghij	24.00 ± 1.41ghij	53.00 ± 1.73e
N29	*Didymella bellidis*	22.00 ± 1.41bcdef	26.00 ± 2.16efghij	55.00 ± 1.89de
N31	*Periconia* spp.	7.50 ± 0.96j	22.00 ± 2.16hij	54.50 ± 2.87de
N33	*Didymella bellidis*	21.00 ± 1.29bcdefg	31.50 ± 2.22defghi	57.50 ± 1.50bcde
N34	*Aspergillus* spp.	9.50 ± 0.96ij	29.50 ± 2.75defghi	56.50 ± 1.71cde
N36	*Fusarium fujikuroi*	25.00 ± 2.08abcd	36.00 ± 2.94bcde	57.00 ± 1.73cde
N41	*Colletotrichum coccodes*	13.50 ±1.50ghij	32.00 ± 2.94cdefgh	55.00 ± 1.29de
N43	*Aspergillus fumigatus*	18.50 ± 0.65bcdefgh	36.00 ± 1.08bcde	53.30 ± 1.25e
N44	*Purpureocillium lilacinum*	16.00 ± 0.82efghi	33.00 ± 1.08bcdefg	52.30 ± 1.65e
	Control	0.0 ± 0.00k	0.0 ± 0.00k	0.50 ± 0.29f
	P-Value	<.0001	<.0001	<.0001

Means with different letter(s) in a column are statistically different according to Tukey's Honestly Significant difference (HSD) test at P≤0.05. Every value (n = 4) is the average of four replicates.

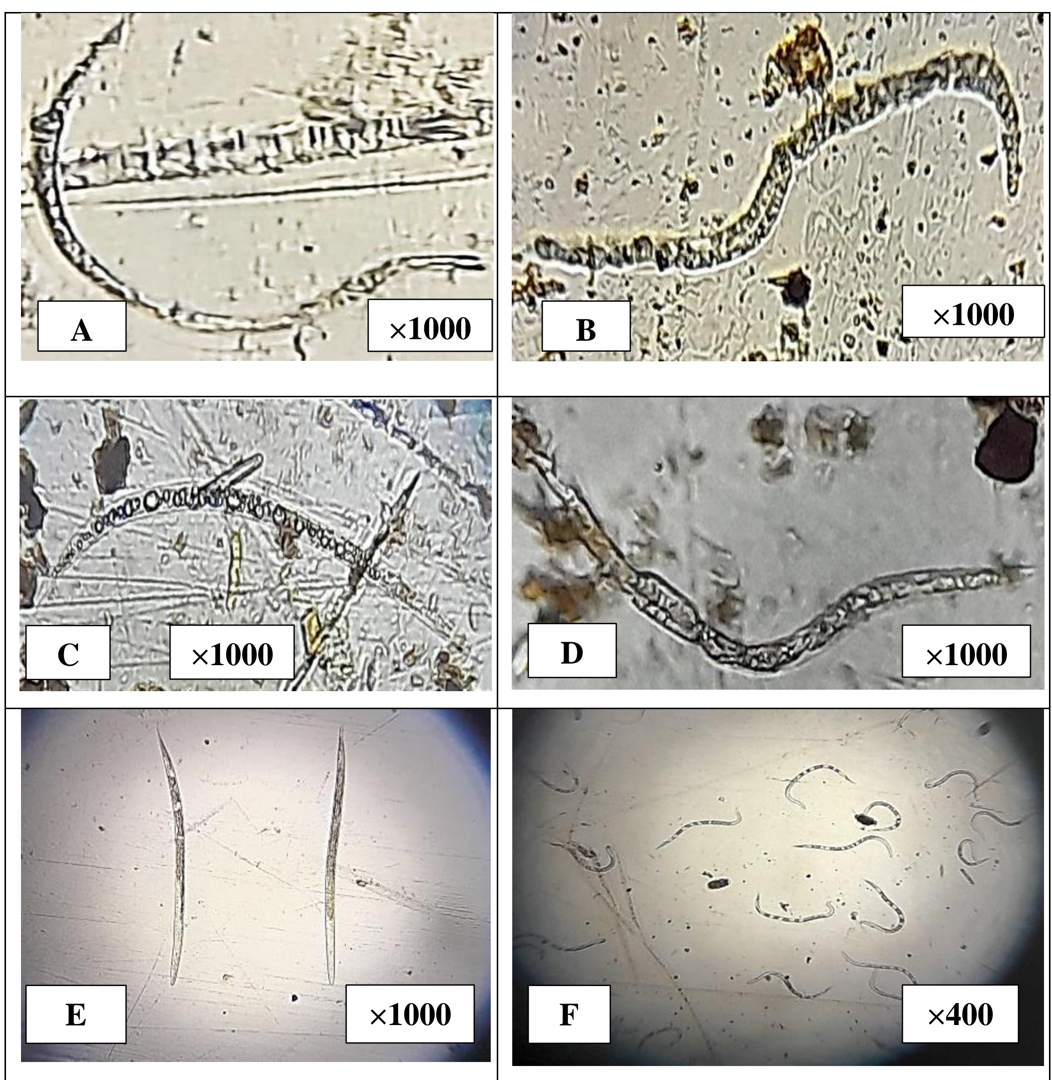


Plate 4.1: Appearance of RKN second stage juveniles after exposure to endophytic fungi *in-vitro*
A= J2 exposed to *Aspergillus* spp. filtrate; **B**; **C**; and **E**= J2 exposed to *Colletotrichum* spp. filtrate; **D**= J2 exposed to *Fusarium* spp. filtrate and **F**= Live RKN J2s in control test. **Source:** Own photos

The fungal isolates' efficacy against J2s of RKN increased with a corresponding increase in the time of exposure to endophytic fungi in both experiments (Figure 4.1).

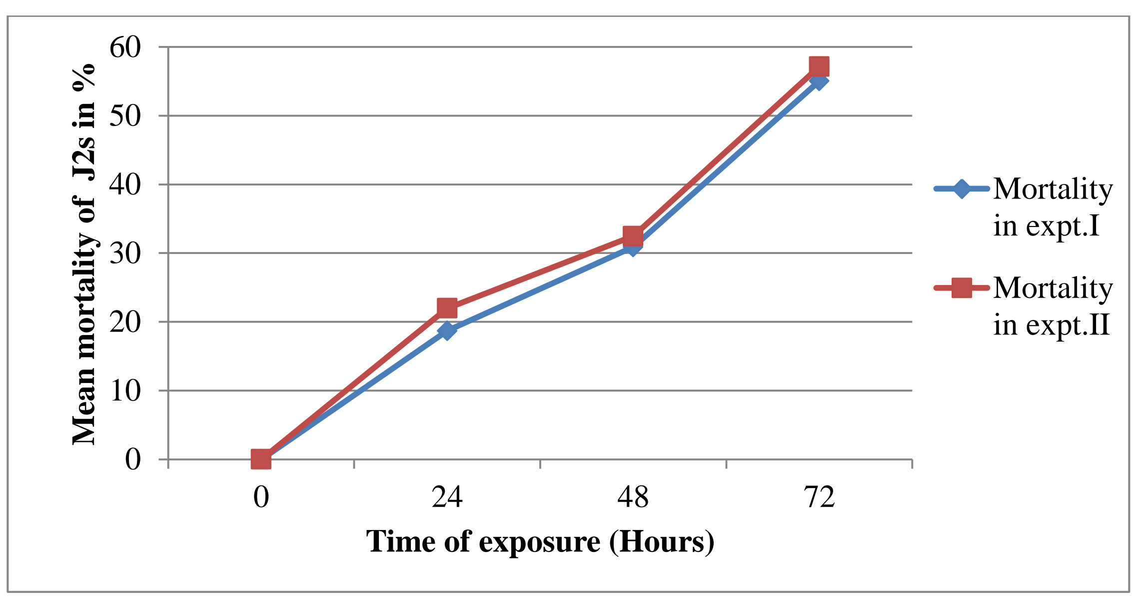


Figure 4:1: Mean mortality of J2s at different times of exposure to fungal endophytes *in vitro*

Regression analysis indicates a positive linear association between mortality of J2s and the time of exposure (Figure 4.2). On further analysis, mortality of J2s was found to have a correlation that was positive and significant ($r=0.91$, $P \leq 0.05$) with time of exposure *in vitro*.

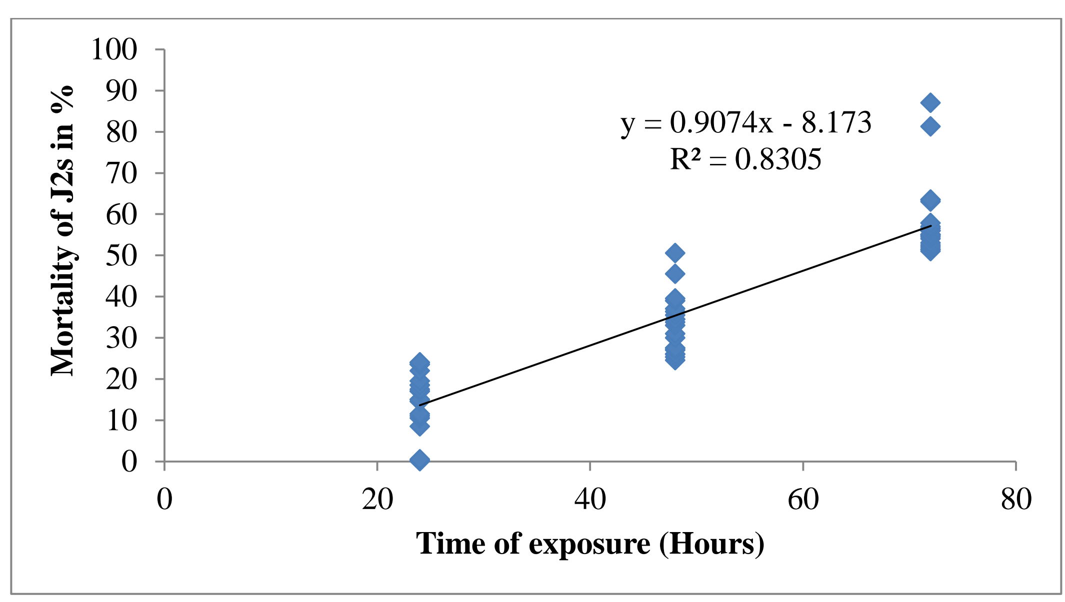


Figure 4:2: Relationship between time of exposure and mortality of J2s *in vitro*

4.2 Identification of endophytic fungal isolates

A total of forty four endophytic fungi were recovered in Nyandarua County from roots of tree tomato that were healthy and eggs of RKN. They had members in, *Colletotrichum* spp. (*C. nigrum*; *C. coccodes*); *Fusarium* spp. (*F. oxysporum*, *F. oxysporum* f.s.p *ciceris*, *F. solani*. *F. fujikuroi*); *Aspergillus* spp. (*A. oerlinghausensis*, *A. ustus*, *A. fumigatus*); *Microsphaeropsis* sp. (*M. arudinis*); *Penicillium* spp. (*P. onobense*, *P. janthinellum*); *Rhizoctonia* spp. (*R. solani*); *Didymella* spp. (*D. bellidis*); *Periconia* sp., and *Purpureocillium* spp. (*P. lilacinum*). Five genera were found in the roots of tree tomato while four genera (*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *purpureocillium* spp.) were found in RKN eggs. *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. were found both in the roots and eggs of RKN while *Purpureocillium* spp. only occurred in eggs of RKN. In roots, *Fusarium* spp. had the

highest absolute frequency of occurrence of 37 % followed by *Penicillium* spp., (22 %), *Colletotrichum* spp., (13 %), *Aspergillus* spp., (16 %) and others (12 % = *Microsphaeropsis* spp., *Rhizoctonia* spp., *Didymella* spp., and *Periconia* spp.,) as shown in figure 4.3. Isolates with lower frequencies of occurrence in roots were classified together as others.

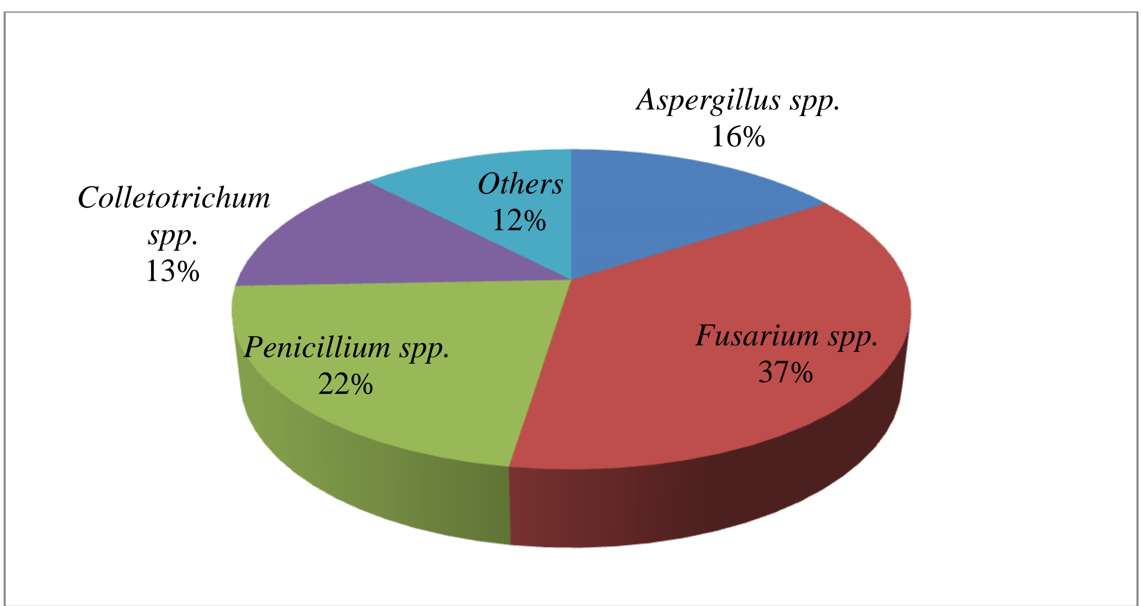


Figure 4:3: Distribution of endophytic fungi isolated from roots of tree tomato
Others are: *Microsphaeropsis* spp., *Rhizoctonia* spp., *Didymella* spp., and *Periconia* spp.

In RKN eggs, *Purpureocillium* spp. was highest with 34% frequency seconded by *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. at 22 % each (Figure 4.4).

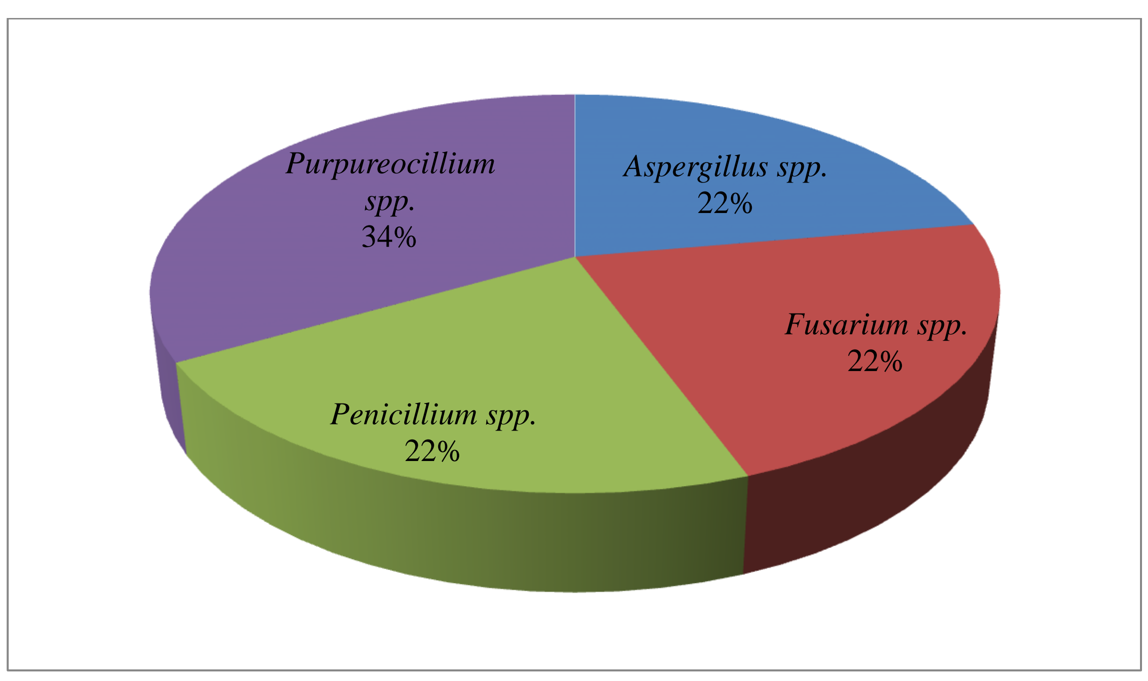


Figure 4:4: Occurrence of endophytic fungi from eggs of RKNs on tree tomato

4.2.1 Morphological descriptions of fungal conidia

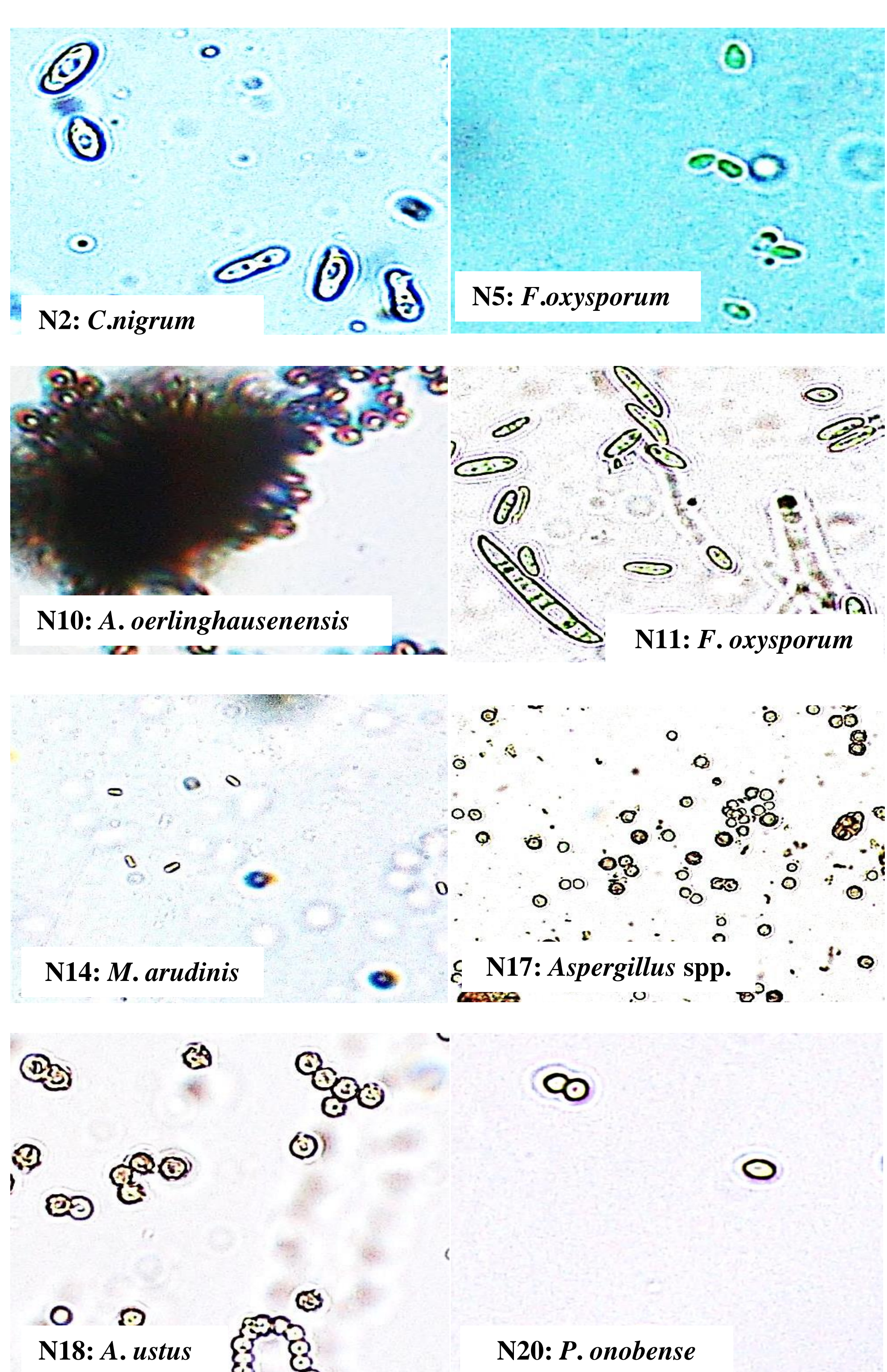
Based on endophytic fungi being effective against RKN J2s mortality, twenty isolates were identified using conidia characteristics under the microscope (Table 4.3 and Plate 4.2).

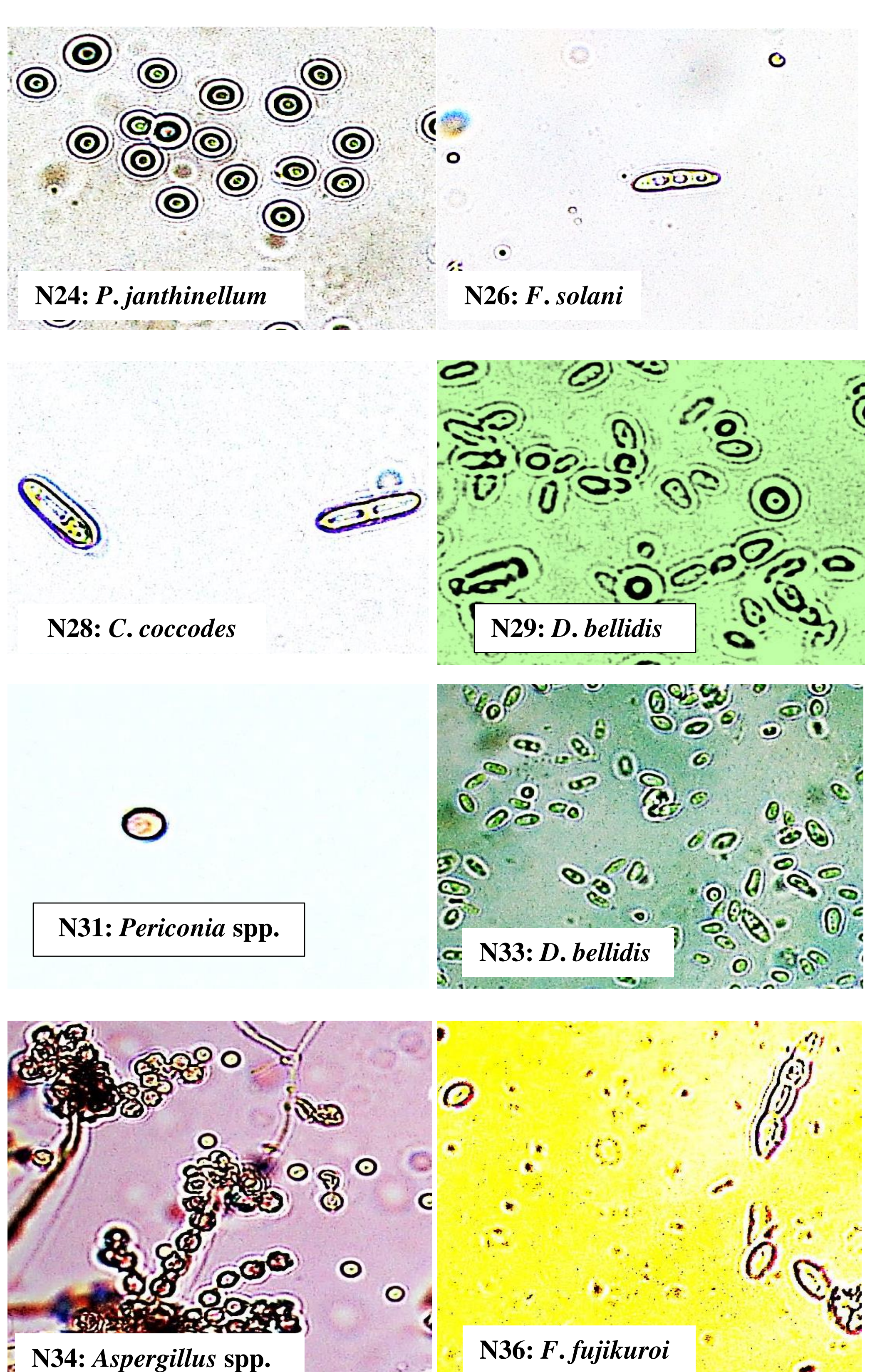
Table 4.3: Conidia characteristics of fungal isolates

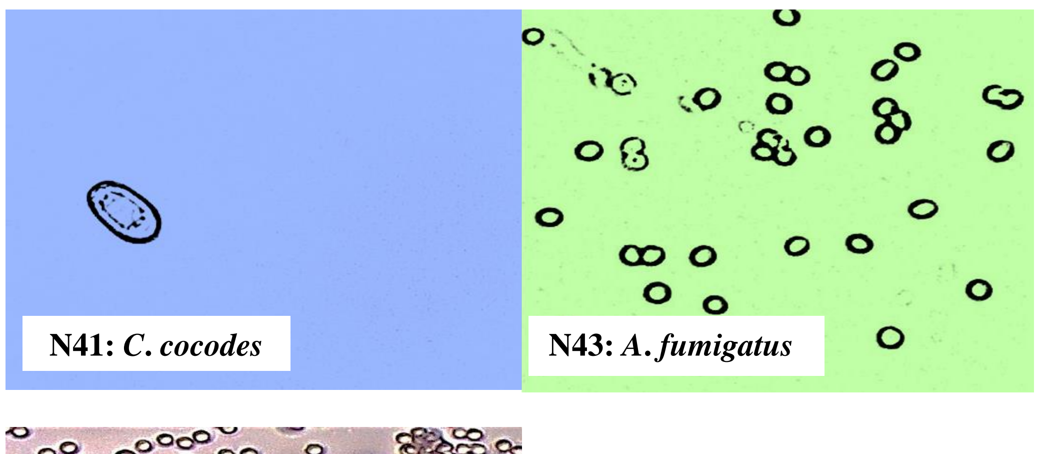
Fungus	Conidia shape and appearance	Size of conidia
Colletotrichum nigrum	Conidia are smooth, narrow hyaline, cylindrical, slightly constricted at the center and aseptate with one end being smaller.	20.2 µm to 27.3 µm by 3.2 µm to 4.8 µm (mean 23.75 by 4.0 µm) Plate 4.2 N2
C. coccodes	Smooth, broad, hyaline, cylindrical, straight, aseptate conidia that is rounded at ends and slightly sunken at the center.	20.0-22.1µm by 5.0-6.9 µm (mean 21.05 by 5.95 µm) Plate 4.2 N28 and N41
Fusarium oxysporum	Microconidia present and numerous appearing singly, ovoid and aseptate. Macroconidia are slightly curved but with ends being almost pointed, thin walled, ellipsoidal and kidney shaped with 3-5 septations.	Microconidia 10.9-18.0 µm by 3.2-6.8 µm (mean 14.45 by 5.0 µm) Macroconidia:20.2-47.1 µm by 4.5-10.5 µm (mean 33.65 by 7.5 µm) Plate 4.2 N5 and N11
Fusarium solani	Microconidia are oval and hyaline with no septa or with up to 2 septation; Macroconidia are slightly curved,	Microconidia are 4-9 µm - 7.11 µm by 3.0-5.1 µm (mean 6.01 by 4.05 µm) Macroconidia are 12.0-35.0

	broad with 3-5 septations.	µm by 2.3-4.9 µm (mean 23.5 by 3.6 µm) Plate 4.2 N (Plate 4.2 N26)
Fusarium fujikuroi	Macroconidia are sickle shaped to nearly straight and narrow at both ends and with 0-5 septa. Microconidia are 1-2 celled, oval and in chains.	Macroconidia are 20.3-80.0 by 2.1-6.3 (mean 50.1 by 4.2 µm) Microconidia are 6.1-24.9 by 2.0-4.1 (mean 15.2 by 3.5 µm) Plate 4.2 N36)
Rhizoctonia solani	Conidia absent	Conidia absent
Microsphaeropsis arudinis	Conidia are one celled, short, cylindrical with both ends round.	3.0-4.2 µm by 1.21- 1.51 µm (mean 3.6 by 1.36 µm) Plate 4.2 N14
Aspergillus oerlinghausenensis	Small conidia making chains from an enlarged cell. Conidia are globose to ellipsoidal, smooth to finely roughened.	2.0 µm to3.1 µm by 2.6 µm to 3.2 µm (mean 2.55 by 2.9 µm) Plate 4.2 N10
Aspergillus ustus	Conidia are globose to ellipsoidal, brown with tiny warty ornamentations.	3.5 µm to 5.1 µm by 3.0 µm to 4.5 µm (mean 4.3 by 3.75 µm) Plate 4.2 N18

Aspergillus fumigatus	Conidia are in chains, elliptical, globose and oblong.	2.1-23.2 by 2.3-3.5 (mean 2.2 by 3.4 µm) Plate 4.2 N43
Penicillium onobense	Conidia produced in chains from the phiallide tips.	2.21 µm to 2.2 µm by 2.2 to 4.1 (mean 2.21 by 3.15 µm) Plate 4.3 N20
Penicillium janthinellum	Conidia are roundish to elliptical.	2.1-4.2 µm by 2.0-4.0 µm (mean 6.3 by 3.0) Plate 4.2 N24
Didymella bellidis	Conidia are aseptate and elliptical, hyaline and unicellular.	3.0-4.1 µm by 2.0-2.6 µm (mean 3.55 by 2.3 µm) Plates 4.2 N29 and N33
Periconia sp.	Conidia are spherical, dark brown with thick walls and aseptate.	4.4 µm to 6.1 µm by 4.1 µm to 6.2 µm (mean 5.25 by 5.15 µm) Plate 4.2 N31
Purpureocillium lilacinum	Conidia are elliptical with smooth wall but slightly rough hyaline with purple hue.	2.6-3.0 µm by 2.0-2.3 µm (mean 2.8 by 2.15 µm) Plate 4.2 N44


N2: C.nigrum
N5: F.oxysporum
N10: A. oerlinghausenensis
N11: F. oxysporum
N14: M. arudinis
N17: Aspergillus spp.
N18: A. ustus
N20: P. onobense


N24: P. janthinellum
N26: F. solani
N28: C. coccodes
N29: D. bellidis
N31: Periconia spp.
N33: D. bellidis
N34: Aspergillus spp.
N36: F. fujikuroi



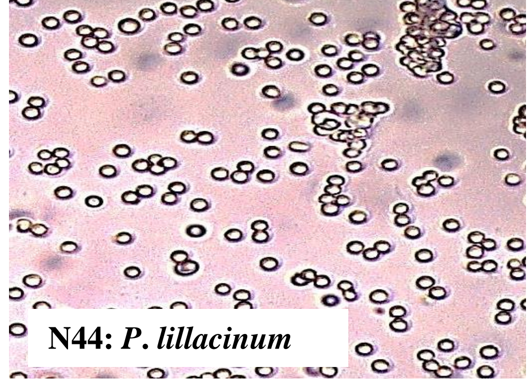


Plate 4.2: Conidia of fungal isolates

4.2.2 Molecular identification of endophytic fungi from tree tomato

4.2.2.1 PCR amplification of genomic DNA

Out of the 20 isolates that exhibited ≥ 50 % efficacy on mortality of J2s, eighteen were identified by DNA sequencing. Isolate N17 could not be sequenced probably due to low DNA yield while isolate N34 was sequenced but could not be identified due to the high level of ambiguities in its sequence. To validate the morphological identification of the endophytic fungal isolates, universal primers targeting the ITS1 and ITS4 genes of rDNA were used for identification. The amplified ITS region produced 500-600 base pairs (Figure 4.5).

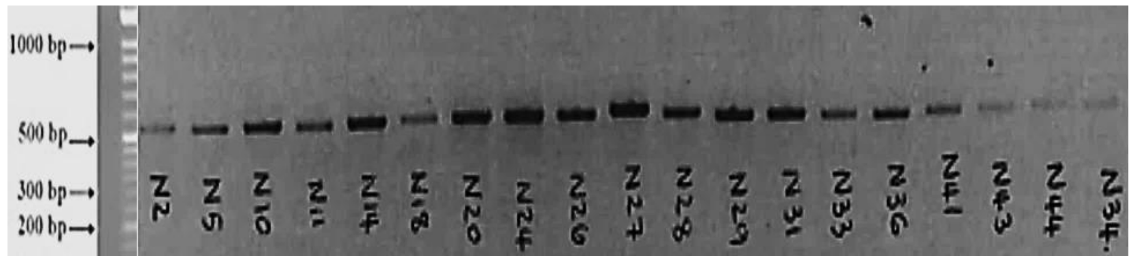


Figure 4:5: Gel electrophoresis of amplified PCR for ITS region of endophytic fungi
N2= *Colletotrichum nigrum*, **N5**=*Fusarium oxysporum*, **N10**=*Aspergillus oerlinghausenensis*, **N11**= *F. oxysporum*, **N14**= *Microsphaeropsis arudins*, **N18**= *A. ustus*, **N20**= *Penicillium onobense*, **N24**= *P. janthinellum*, **N26**= *Fusarium solani*, **N27**= *Rhizoctonia solani*, **N28**= *C. coccodes*, **N29**= *Didymella bellidis*, **N31**= *Periconia* sp., **N33**= *D. bellidis*, **N36**= *F. fujikuroi*, **N41**= *C. coccodes*, **N43**= *A. fumigatus* and **N44**= *Purpureocillium lilacinum*

4.2.2.2 Phylogenetic identification of tree tomato endophytic fungal isolates

Molecular identification of endophytic fungi from tree tomato was inferred on 18 ITS sequences (Appendix 7). After sequencing, *Fusarium* spp. had four isolates, *Colletotrichum* spp. and *Aspergillus* spp. had 3 isolates each while *Penicillium* spp. and *Didymella* spp. had two isolates each. *Microsphaeropsis* spp., *Rhizoctonia* spp., *Periconia* spp., and *Purpureocillium* spp. had one sequence each as shown in Table 4.4. The closest percentage similarity between isolates from this study and those from GeneBank ranged between 93 to 100 % with 96 – 100 % query cover as shown in Table 4.4.

Table 4.4: GeneBank accession numbers obtained for isolates from this study and NCBI Accession reference numbers used in identification of species from ITS sequences

ITS Sequences					
Isolates	**GeneBank accession numbers from this study**	**Highest identity to**	**Accession ref. numbers from GeneBank**	**% Similarity**	**Query cover (%)**
N2	OQ953744	*Colletotrichum nigrum*	ON102024.1	100	98.63
N28	OQ953754	*C. coccodes*	GU93588.1	100	99.13
N41	OQ953758	*C. coccodes*	KU821175.1	99	99.83
N5	OQ953745	*Fusarium oxysporum*	MKO74845.1	100	100
N11	OQ953747	*F. oxysporum*	MT420611.1	98	100
N26	OQ953752	*Fusarium solani*	KP265362.1	98	99.64
N36	OQ953757	*Fusarium fujikuroi*	MH084746.1	100	96.09
N10	OQ953746	*Aspergillus oerlinghausenensis*	NR-138362.1	100	99.83
N18	OQ953749	*A. ustus*	KF534498.1	100	100
N43	OQ953759	*Aspergillus fumigatus*	MT558940.1	100	98.66
N14	OQ953748	*Microsphaeropsis arudins*	EF094554.1	93	99.66
N20	OQ953750	*Penicillium onobense*	MK450706.1	97	99.33
N24	OQ953751	*P. janthinellum*	KM268648.1	99	99.33
N27	OQ953753	*Rhizoctonia solani*	MT747193.1	99	96.39
N29	OQ953755	*Didymella bellidis*	MN274963.1	99	99.63
N31	OQ953761	*Periconia* sp.	MT420640.1	99	99.65
N33	OQ953756	*D. bellidis*	MN202254.1	97	99.25
N44	OQ953760	*Purpureocillium lilacinum*	MK724002.1	100	98.31

4.2.2.3 Phylogenetic relationship among fungal endophytes

The phylogeny tree was built using 18 ITS sequences of endophytic fungi from tree tomato to determine their evolutionary closeness. The tree was built in MEGA 11 by clustalW using Neighbor-Joining (NJ) method with 1000 bootstrap replicates to determine their support for phylogenetic branching or clades. Alignment and phylogenetic analysis on the sequences produced two main clades with clade 1 having two sub-clades separated with different bootstrap values (Figure 4.6).

The alignment of sequences and phylogenetic analyses produced two clades separated with different bootstrap support values (Figure 4.6). Clade I is composed of seventeen isolates supported by 51-100 % bootstrap values. This clade has two sub clades with the first sub-clade comprising *Fusarium* sp., *Purpureocillium* sp., *Colletotrichum* sp., *Aspergillus* sp., and *Penicillium* sp. Isolates N36, N11 and N5 are clustered together with *F. oxysporum* GeneBank species. Isolates N36 is clustered with *F. fujikuroi*. Isolate N26 is clustered with *F. solani* as sister taxons with 100 % bootstrap support value while isolate N44 is clustered together with *Purpureocillium lilacinum* as sister taxons with 100 % bootstrap support value. Isolates N2, N41 and N28 are clustered together with *Colletotrichum nigrum* and *C. coccodes* with 73-99 % bootstrap support values. Isolate N18 is clustered with *Aspergillus ustus* as sister taxons with 100 % bootstrap value while isolate N43 and N10 are clustered together with *A. fumigatus* and *A. oerlinghausenensis* respectively with 86-99 % bootstrap support values. Isolate N20 is clustered together with *Penicillium onobense* as sister taxons while isolate N24 is clustered with *P. janthinellum* as sister taxons with 92-99 % bootstrap values. Isolate N29 and N33 are clustered with *Didymella bellidis*. In the

second sub-clade, isolate N14 is clustered with *Microsphaeropsis arudinis* while N31 is clustered with *Periconia* sp. with 96 % bootstrap support value.

In the second clade, isolate N27 is clustered with *Rhizoctonia solani* with 100 % bootstrap support value (Figure 4.6).

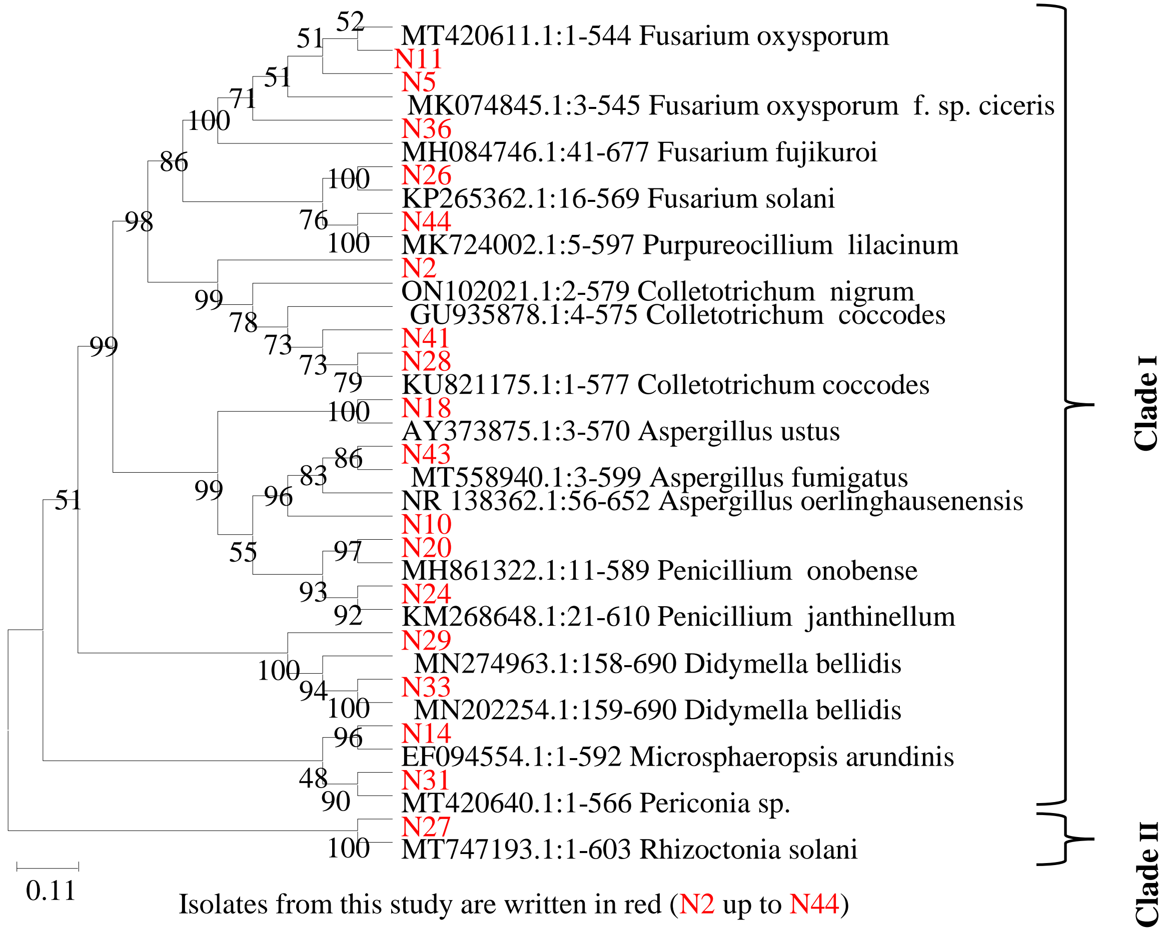


Figure 4:6: Neighbor-Joining phylogenetic tree indicating the relationship between endophytic fungal based on ITS sequences.

Analyses were performed using 1000 bootstrap replications. Numbers near each branch show replicate trees in percentage of by which the related taxa are clustered together in the bootstrap test (1000 replicas) values in each clade.

4.2.2.4 Nucleotide frequencies (%)

The average nucleotide frequencies for the 18 endophytic fungal isolates were 24.0 for T, 27.6 (C), 23.1 (A) and 25.3 (G) as depicted in Table 4.5.

Table 4.5: Nucleotide frequencies

Code	Isolate	Thymine	Cytosine	Adenine	Guanine	Total
N2	*Colletotrichum nigrum*	24.5	26.4	23.7	25.4	583
N5	*Fusarium oxysporum f. sp. ciceris*	24.1	26.0	27.1	22.8	543
N10	*Aspergillus oerlinghausensis*	21.2	30.3	20.1	28.4	598
N11	*Fusarium oxysporum*	24.3	25.9	26.7	23.1	555
N14	*Microsphaeropsis arundinis*	25.4	28.2	21.3	25.1	634
N18	*Aspergillus ustus*	21.5	29.8	19.8	28.9	567
M20	*Penicillium onobense*	21.8	29.7	19.9	28.7	593
N24	*Penicillium janthinellum*	20.3	31.4	18.8	29.4	595
N26	*Fusarium solani*	21.4	28.5	25.8	24.4	562
N27	*Rhizoctonia solani*	33.1	20.2	26.7	20.0	610
N28	*Colletotrichum coccodes*	23.6	27.3	23.8	25.3	572
N29	*Didymella bellidis*	27.0	24.3	25.2	23.5	622
N31	*Periconia sp.*	24.4	29.2	23.0	23.3	996
N33	*Didymella bellidis*	28.4	24.4	24.0	23.3	550
N36	*Fusarium fujikuroi*	24.8	25.7	25.9	23.7	630
N41	*Colletotrichum coccodes*	23.9	26.8	23.7	25.6	578
N43	*Aspergillus fumigatus*	22.9	29.1	19.4	28.5	597
N44	*Purpureocillium lilacinum*	17.7	33.1	22.8	26.5	593
	Average	**24.0**	**27.6**	**23.1**	**25.3**	**590.6**

4.2.2.5 Tajma's neutrality test

Tajmas's neutrality test for 18 nucleotide sequences of endophytic fungal isolates are as shown in Table 4.6. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). The final dataset had a total of 426

positions. The evolutionary analyses were conducted in MEGA11. With a Tajma's test score of 2.084823, the diversity of nucleotides from aligning the 18 isolate sequences recorded 0.227487 (Table 4.6).

Table 4.6: Neutrality (Tajma's) test for endophytic fungal sequences

m	S	p_s	Θ	π	D
36	259	0.607981	0.146615	0.227487	2.084823

Abbreviations: The Tajma test statistic is D. The other variables are: m = number of sequences, n = total number of sites, S = number of segregating sites, $ps = S/n$, $\Theta = ps/a1$, π = nucleotide diversity

4.2.2.6 Estimate of the nucleotide substitution matrix's maximum probability

Each entry's result indicates the likelihood of transitioning from one base (row) to another base (column). The rates of various transitional substitutions are displayed in bold, whereas the rates of transversional substitutions are displayed in italics (Table 4.7). The relative values of concise susbstitution should be taken into account when evaluating them.

Table 4.7: Substitution of nucleotides

	A	**T/U**	**C**	**G**
A	-	*6.34*	*7.30*	**8.37**
T/U	*6.12*	-	**16.63**	*6.69*
C	*6.12*	**14.44**	-	*6.69*
G	**7.66**	*6.34*	*7.30*	-

Pattern and rates of substitution were estimated under the Tamura-Nei (1993) model

4.3 Compatibility of Velum® Prime with endophytic *Colletotrichum nigrum* and *Trichoderma asperellum* isolates against J2s of RKN on tree tomato

4.3.1 Effect of Velum® Prime on colony growth of *Colletotrichum nigrum* and commercial *Trichoderma asperellum in vitro*

Regarding to isolated endophytic *C. nigrum*, there was no significant change ($P \geq 0.05$) in the colony diameter when exposed to Velum® Prime compared to the untreated control (*C. nigrum* alone) as shown in Table 4.8 (experiment I and II) and plate 4.3. Commercial *T. asperellum* growth did not differ statistically from the untreated control (*T. asperellum* only) on exposure to Velum® Prime in experiment I and II as shown in Table 4.8 and plate 4.3.

Table 4.8: Effect of Velum® Prime on *Colletotrichum nigrum* and *Trichoderma asperellum* growth (colony diameter)

	Expt. I	**Expt. II**
Treatment	**% Inhibition**	**% Inhibition**
C. nigrum + Velum® Prime	1.00 ± 0.79a	1.02 ± 0.73a
C. nigrum alone (control)	0.00 ± 0.00a	0.00 ± 0.00a
Commercial *T. asperellum*+ Velum® Prime	1.01 ± 0.58a	0.99 ± 0.33a
Commercial *T. asperellum* alone (control)	0.00 ± 0.00a	0.00 ± 0.00a
P-value	0.29	0.27

Values are means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column having the same letter are not statistically different

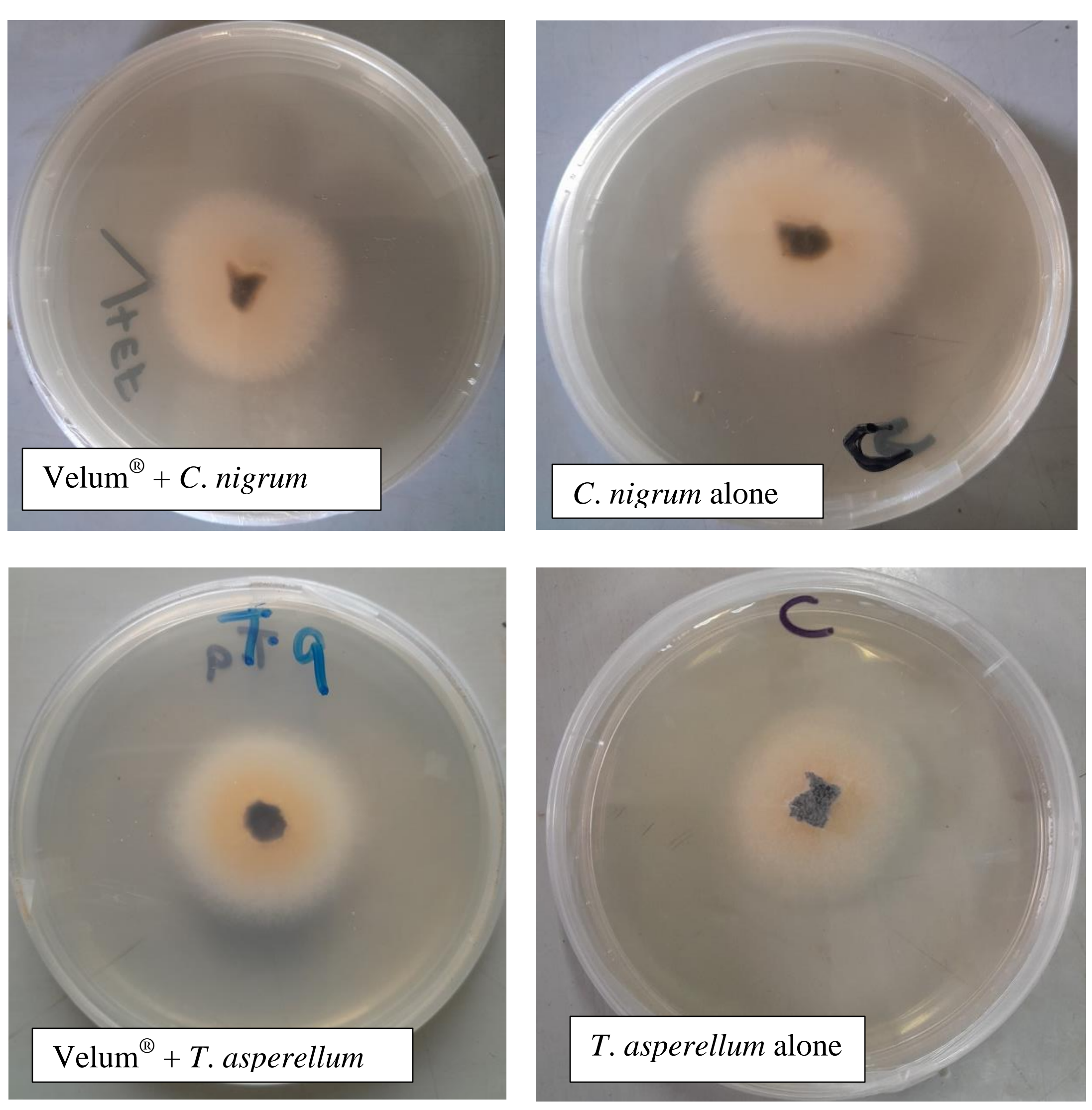


Plate 4.3: Effect of Velum® Prime on colony diameter of endophytic *Colletotricum nigrum* and commercial *Trichoderma asperellum*

4.3.2 Efficacy of *Colletotrichum nigrum* and *Trichoderma asperellum* combined with Velum® Prime against RKNs on tree tomato in the greenhouse

i) Effect on population of second stage juveniles of RKN in soil and roots

The soil population of J2s differed significantly (P≤0.05) among the treatments (Tables 4.9 and 4.10). Nematode-inoculated plants treated with *C. nigrum* + Velum® Prime had the lowest population of J2s per 200 cc of dry soil followed by those treated

with *T. asperellum* + Velum® Prime and Velum® Prime alone, respectively (Tables 4.9 and 4.10). All treatments significantly reduced nematode populations in the rhizospheric soil compared to the positive control.

The second stage juvenile populations (J2s) in roots were statistically different ($P \leq 0.05$) between all treatments. Nematode-inoculated plants with *C. nigrum* + Velum® Prime treatment had the lowest J2s per 5g of dry roots followed by those treated with *T. asperellum* + Velum® Prime and Velum® Prime alone, respectively (Tables 4.9 and 4.10). All treatments significantly reduced RKN J2 populations in roots as compared to the positive control.

ii) Effect on RKN reproduction factor

The RKN reproduction factor was statistically different ($P \leq 0.05$) between different treatments as shown in Tables 4.9 and 4.10. The *C. nigrum* + Velum® Prime treatment significantly reduced RKN reproduction factor followed by *T. asperellum* + Velum® Prime and Velum® Prime alone relative to positive control.

Table 4.9: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test I

Treatment	**J2s/200cc dry soil**	**J2s/5g dry roots**	**Rf**
Colletotrichum nigrum + RKN J2s	549.17 ± 24.85b	186.25 ± 23.04b	0.28 ± 0.01b
Trichoderma asperellum + RKN J2s	580.83 ± 18.00b	198.75 ± 12.14b	0.29 ± 0.01b
C. nigrum + Velum® Prime + RKN J2s	215.83 ± 9.26d	45.00 ± 28.50d	0.11 ± 0.01cd
T. asperellum + Velum® Prime + RKN J2s	226.67 ± 5.27d	57.50 ± 12.50d	0.14 ± 0.02cd
Velum® Prime + RKN J2s	303.27 ± 6.21c	111.35 ± 9.05c	0.17± 0.03c
Positive control (RKN J2s alone)	3223.33 ± 30.46a	516.25 ± 30.44a	1.61 ± 0.02a
Negative control (untreated/uninoculated)	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
P-value	<.0001	<.0001	<.0001

The values are means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column having different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The final (Pf) to initial (Pi) nematode populations ratio constituted nematode reproduction factor (**Rf**). Negative control = untreated control without RKN J2s.

Table 4.10: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test II

Treatment	J2s/200cc dry soil	J2s/5g dry roots	Rf
Colletotrichum nigrum + RKN J2s	542.50 ± 12.96b	182.50 ± 31.52b	0.27 ± 0.01b
Trichoderma asperellum + RKN J2s	574.17 ± 31.56b	191.25 ± 4.73b	0.29 ± 0.02b
C. nigrum + Velum® Prime + RKN J2s	208.33 ± 12.29d	41.25 ± 4.27d	0.10 ± 0.01cd
T. asperellum + Velum® Prime + RKN J2s	219.17 ± 9.44d	51.23 ± 2.39d	0.11 ± 0.01cd
Velum® Prime + RKN J2s	312.00 ± 14.07c	107.25 ± 9.08c	0.15 ± 0.02c
Positive control (RKN J2s alone)	3211.67 ± 113.81a	498.75 ± 14.63a	1.610.06a
Negative control (untreated and uninoculated)	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
P-value	<.0001	<.0001	<.0001

The values are means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column having different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The final (Pf) to initial (Pi) nematode populations ratio constituted nematode reproduction factor (**Rf**). Negative control = untreated control without RKN J2s.

Regression analysis indicated a positive linear association between nematode reproduction factor (Rf) and RKN J2 populations (Figure 4.7). A further investigation showed that the Rf and soil J2 populations correlated positively (r=1, P=0.05).

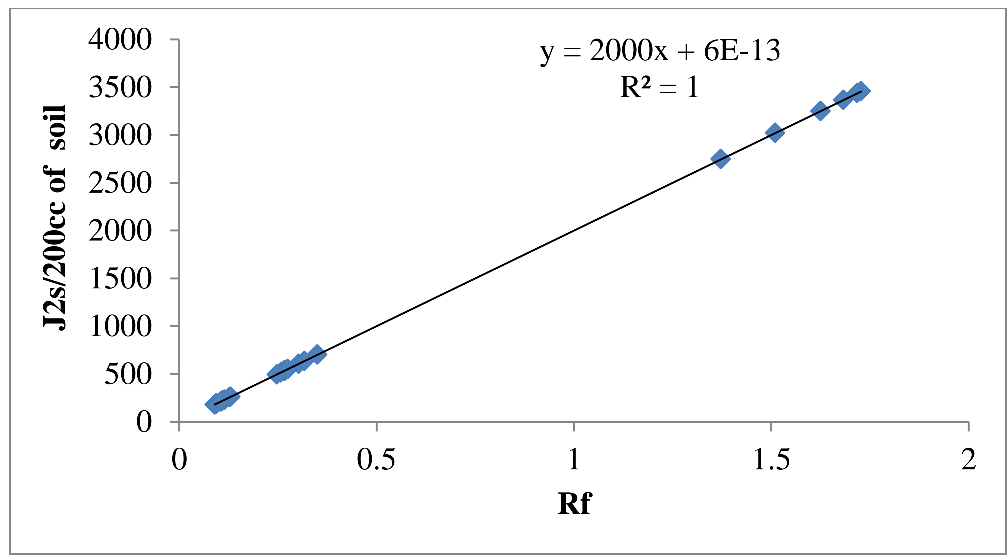


Figure 4:7: Regression analysis between nematode reproduction factor and populations of J2s in the soil (Greenhouse experiment)

iii) Effect on gall and egg mass indices

There was an existence of statistical difference (P≤0.05) in terms of indices on gall and egg masses among the treatments (Table 4. 11). Plants treated with *C. nigrum* + Velum® Prime had the lowest gall and egg mass indices followed by those treated with *T. asperellum* + Velum® Prime and Velum® Prime alone as shown in Table 4. 11. The least gall and egg mass indices reduction was shown in *T.asperellum* treatment.

Table 4.11: Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum® Prime on indices of galling and egg masses on tree tomato in the greenhouse test I and II

	Greenhouse test I		Greenhouse test II	
Treatment	**Gall index**	**Egg mass index**	**Gall index**	**Egg mass index**
Colletotrichum nigrum + RKN J2s	3.67 ± 0.21b	2.75 ± 0.25bc	3.83 ± 0.09b	3.00 ± 0.00b
Trichoderma asperellum + RKN J2s	3.83 ± 0.17b	3.25 ± 0.25b	3.67 ± 0.56b	3.33 ± 0.50b
C. nigrum + Velum® Prime + RKN J2s	1.50 ± 0.43cd	1.50 ± 0.29cd	1.00 ± 0.45d	1.17 ± 0.31d
T. asperellum + Velum® Prime + RKN J2s	2.00 ± 0.43cd	2.00 ± 0.00c	1.50 ± 0.43d	1.33 ± 0.33d
Velum® Prime + RKN J2s	2.25± 0.51 c	2.05± 0.01c	2.00 ± 0.50c	2.00 ± 0.00c
Positive control (RKN J2s alone)	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a
Negative control (untreated/uninoculated)	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00e
P-value	<.0001	<.0001	<.0001	<.0001

The values are means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different. Negative control = untreated control without RKN J2s. Gall and egg mass indices were scored on a scale of 0-5 (Quesenberry *et al*., 1989) as indicated in Table 3.1.

iv) Effect on plant shoot height

The heights of plants in various treatments significantly differed ($P \leq 0.05$) from one another. The heights of uninoculated (without nematodes) plants treated with *C. nigrum* and *Trichoderma asperellum* as standalone treatments had statistically higher ($P \leq 0.05$) shoots than those of the negative control (without nematodes and without treatments) as shown in Tables 4.12 and 4.13. The heights of *C. nigrum* + Velum® Prime + J2s, *T. asperellum* + Velum® Prime + J2s, *C. nigrum*+ J2s and *T. asperellum* + J2s treated plants were significantly higher than those of the positive control (J2s alone). The results showed that all treatments increased the height of shoots of tree tomato relative to the positive control (Plate 4.4, Tables 4.12 and 4.13).

Plate 4.4: Differences in growth of tree tomato under different treatments in the greenhouse.

a - h represents treatments where; **a** = inoculated + *Colletotrichum nigrum*; **b** = inoclated + *Trichoderma asperellum*; **c** = inoculated + *C. nigrum* + Velum® Prime; **d** = inoculated + *T. asperellum* + Velum® Prime; **e** = negative control (un-treated and un-inoculated); **f** = Positive control (un-treated and inoculated); **g** = *C. nigrum* alone; and **h** = *T. asperellum* alone

v) Effect on weight of dry roots

The weight of dry roots significantly varied ($P \leq 0.05$) amongst treatments (Tables 4.12 and 4.13). The heaviest weight of dry root was recorded in uninoculated (without J2s) plants treated with *C. nigrum* followed by uninoculated plants treated with *T. asperellum* and negative control (untreated and uninoculated). All uninoculated treated (other treatments other than J2s) plants had significantly higher weights of dry root than those of inoculated treatments. The weights of dry root of inoculated and treated tree tomato plants were significantly higher than those of the positive control (J2s alone). *Colletotrichum nigrum* + Velum® Prime had significantly higher weight of dry root among nematode inoculated treatments relative to the positive control (Tables 4.12 and 4.13).

vi) Effect on weight of dry shoot

There were statistical differences ($P \leq 0.05$) among the weights of dry shoot of plants among the treatments (Tables 4.12 and 4.13). Un-inoculated plants treated with *C. nigrum*, *T. asperellum* and negative control (untreated and uninoculated) had higher weights of dry shoot as compared to nematode inoculated and treated plants. All plants in the treatments without nematodes had higher weights of dry shoot than those of inoculated treatments. The weights of dry shoot of inoculated treatments were higher than those of the positive control (J2s alone). For nematode inoculated plants, *C. nigrum* + Velum® Prime treatment had the highest mean weight of dry shoot followed by *T. asperellum* + Velum® Prime relative to the positive control (Tables 4.12 and 4.13).

Table 4.12: Shoot height (SH), dry root weight (DRW) and dry shoot weight (DSW) of tree tomato treated with *Colletotrichum nigrum* and *Trichoderma asperellum* combined with Velum® Prime in the greenhouse test I

Treatment	SH	DRW	DSW
Colletotrichum nigrum + Velum® Prime + RKN J2s	35.18 ± 0.64c	4.56 ± 0.07b	8.25 ± 1.5b
C. nigrum + Velum® Prime - RKN J2s	43.98 ± 1.49ab	6.84 ±0.65 ab	10.25 ± 0.25ab
Trichoderma asperellum + Velum® Prime + RKN J2s	34.37 ± 1.76c	3.99 ± 0.79c	8.08 ± 0.92b
T. asperellum + Velum® Prime - RKN J2s	43.81 ± 1.44ab	6.72 ± 0.69b	10.13 ± 0.96ab
C. nigrum + RKN J2s	33.22 ± 1.77c	3.11 ± 0.12c	7.87 ± 0.31c
C. nigrum - RKN J2s	46.87 ± 1.41a	7.64 ± 0.16a	11.65 ± 0.43a
T. asperellum + RKN J2s	33.02 ± 0.59c	3.00 ± 0.66c	7.10 ± 0.53c
T. asperellum - RKN J2s	46.53 ± 0.50a	7.31 ± 0.48a	11.22 ± 0.27a
Velum® Prime + RKN J2s	34.01 ± 1.70c	4.14 ± 0.88b	6.99 ± 1.45c
Velum® Prime - RKN J2s	39.10 ± .0 57b	4.85 ± 0.81b	7.03 ± 0.41c
Positive control (RKN J2s alone)	26.08 ± 0.64d	2.03 ± 0.33d	5.09 ± 0.20d
Negative control (untreated and uninoculated)	42.63 ± 1.52ab	6.37 ± 0.64ab	10.02 ± 0.26ab
P-value	<.0001	<.0001	<.0001

The numbers depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different. Negative control = untreated control without RKN J2s.

Table 4.13: Shoot height (SH), dry root weight (DRW) and dry shoot weight (DSW) of tree tomato treated with *Colletotrichum nigrum* and *Trichoderma asperellum* combined with Velum® Prime in the greenhouse test II

Treatment	SH	DRW	DSW
Colletotrichum nigrum + Velum® Prime + RKN J2s	35.67 ± 2.57d	4.20 ± 0.40b	9.19 ± 1.32b
C. nigrum + Velum® Prime - RKN J2s	45.87 ± 1.11b	6.27 ± 0.58ab	11.34 ± 0.50a
Trichoderma asperellum + Velum® Prime + RKN J2s	35.05 ± 2.00d	3.85 ± 0.21b	9.01 ± 1.14b
T. asperellum + Velum® Prime - RKN J2s	45.03 ± 1.10b	6.23 ± 0.50ab	11.17 ± 0.53a
C. nigrum + RKN J2s	34.92 ± 2.25d	3.09 ± 0.34bc	8.71 ± 1.06b
C. nigrum - RKN J2s	48.67 ± 0.77a	7.15 ± 0.26a	12.14 ± 0.38a
T. asperellum + RKN J2s	33.91 ± 1.15d	2.93 ± 0.24bc	8.64 ± 0.51b
T. asperellum - RKN J2s	48.33 ± 0.98a	7.05 ± 0.39a	11.80 ± 0.56a
Velum® Prime + RKN J2s	34.47 ± 2.21d	4.03 ± 0.38b	6.09 ± 1.12c
Velum® Prime - RKN J2s	40.21 ± 0.99c	4.22 ± 0.40b	7.06 ± 1.09c
Positive control (RKN J2s alone)	29.25 ± 1.91e	1.97 ± 0.20c	5.84 ± 0.43d
Negative control (untreated and uninoculated)	44.50 ± 1.68b	6.15 ± 0.49ab	10.47 ± 0.44ab
P-value	<.0001	<.0001	<.0001

The numbers depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different. Negative control = untreated control without RKN J2s.

4.4 Efficacy of combining *Colletotrichum nigrum*, *Lantana camara* leaf extract and Velum® Prime against Root-knot nematodes on tree tomato

4.4.1 Effect of *Lantana* leaf extract on mortality of RKN J2s *in vitro*

The outcome of this research showed that different concentrations of *Lantana* leaf extract applied alone were able to kill J2s of RKN at the end of 24, 48 and 72 hours of exposure periods (Table 4.14). The numbers of juvenile mortality in varying concentrations of *Lantana* leaf extract after 24, 48, and 72 hours in experiments I and II, respectively, showed statistical difference ($P \leq 0.05$) as indicated in Table 4.14. In both experiments the highest mortality of J2s was seen in the 100 % followed by 75 %, 50 %, and 25 % concentrations in decreasing order. *Lantana* leaf extract at 100 % concentrations caused the highest mortality of J2s in experiment I and II at the end of 24, 48, and 72 hours, respectively.

Table 4.14: Effect of varying concentrations of leaf extract of *Lantana camara* on mortality of RKN J2s *in-vitro*

% Mortality of RKN J2s treated with varying concentrations of *Lantana* leaf extract						
	Experiment I			**Experiment II**		
Concentration of *Lantana* leaf extract	**24 Hours**	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**
25 %	27.00±1.29d	47.00±1.00d	57.0±01.29d	27.00±0.58d	48.00±0.82d	56.50±0.96d
50 %	46.00±2.00c	57.00±1.29c	66.50±0.96c	45.50±0.96c	56.50±0.96c	68.00±1.83c
75 %	58.00±0.96b	67.50±1.00b	78.50±0.96b	56.00±1.15b	70.00±0.82b	80.00±0.82b
100 %	68.00±0.82a	79.00±1.29a	89.00±1.29a	69.00±0.58a	81.00±2.58a	91.500±1.71a
Control(distilled water)	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

The numbers depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different.

On the effect of time, it was observed that the length of time of exposure had a correlation on the rate of mortality of J2s and hence the duration of 72 hours produced the highest mortality of J2s among the concentrations as compared to the control in experiment I and II (Figure 4.8 and Table 4.14).

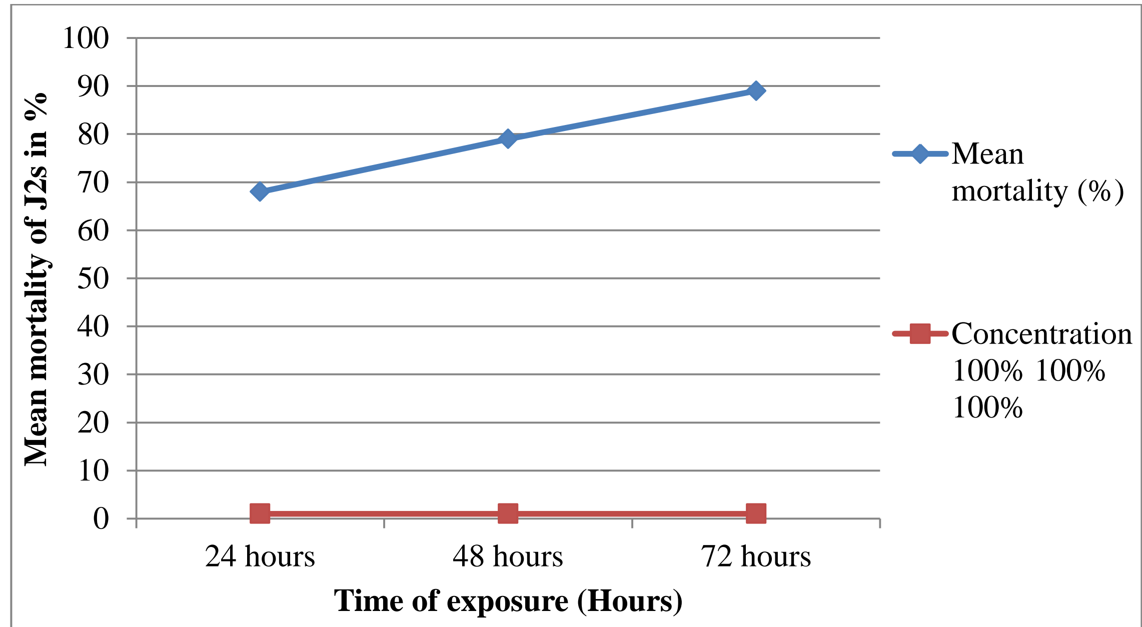


Figure 4:8: A line graph showing the relationship between time of exposure to *Lantana* leaf extract and mortality of J2s *in vitro*

In both experiments, there was a general trend of increasing mortality of J2s with increasing *Lantana* leaf extract concentrations and vice versa (Figure 4.9 and Table 4.14). The 100% concentrations had the highest mortality of J2s compared to other concentrations (Figure 4.10)

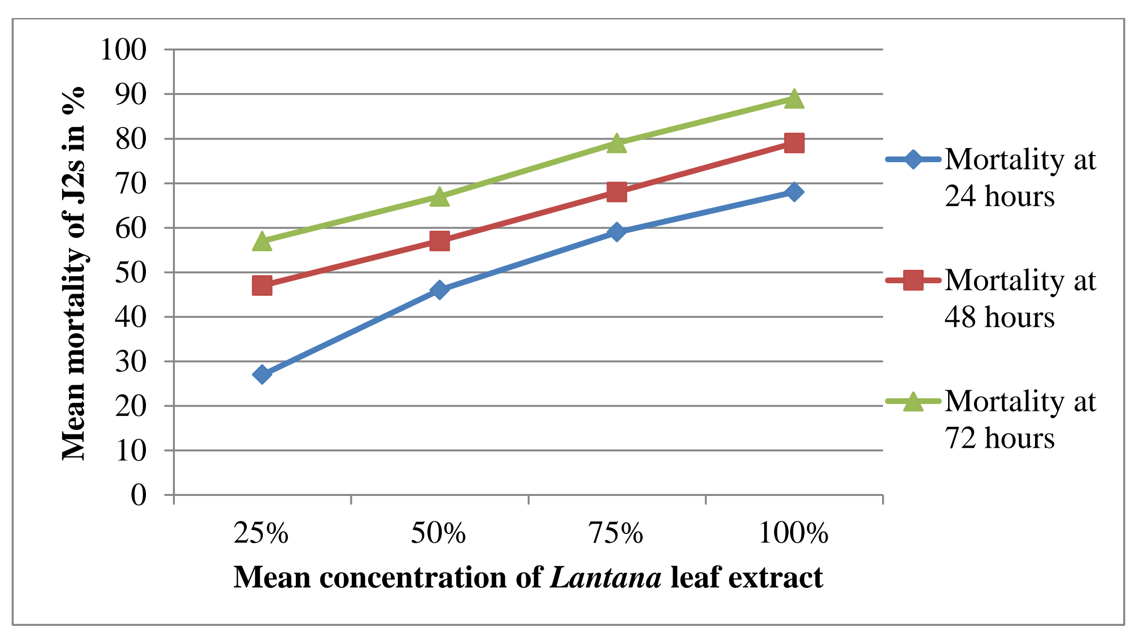


Figure 4:9: Line graphs showing the relationship between various concentrations of *Lantana* leaf extract and mortality of J2s *in vitro*

Regression analysis revealed a positive linear relationship between *Lantana* leaf extract concentrations and the time of exposure. On further analysis, the time of exposure with regard to mortality of J2s was found to be correlated positively (r=0.91, at P≤0.05) with concentration in *in vitro* test I (Figure 4.10) and similar trend was observed in *in vitro* test II.

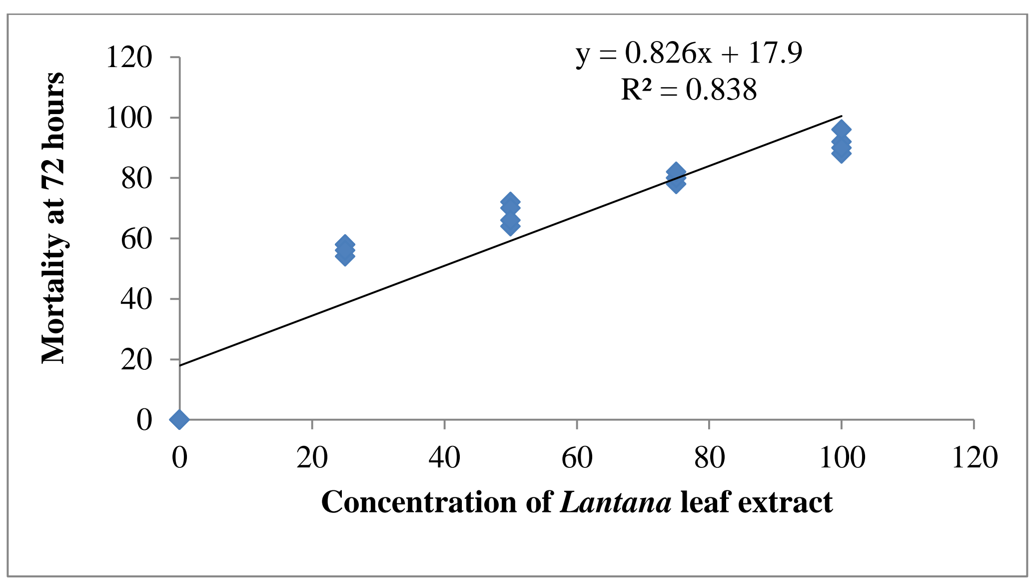


Figure 4:10: Relationship between the concentration of *Lantana* leaf extract and mortality of J2s after exposure for 72 hours *in vitro*

4.4.2 Effect of combining *Lantana* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in vitro*

The various concentrations of *Lantana* leaf extract in combination with *C. nigrum* caused significant mortality of RKN J2s after 24, 48, and 72 hours in experiments I and II respectively (Table 4.15). *Colletotrichum nigrum* (1×10^6 spores/ml) gave the highest ($P \leq 0.05$) mortality of J2s followed by 100 % *Lantana* leaf extract in combination with *C. nigrum* in both experiments at the end of 24, 48, and 72 hours, respectively (Table 4.15).

In comparison the 100 % concentration of *Lantana* leaf extract applied alone had the highest ($P \leq 0.05$) mortalities of J2s in experiment I and II after 72 hours (Table 4.14) as compared to when combined with *C. nigrum* in both experiments after 72 hours of exposure (Table 4.15).

Table 4.15: Efficacy of combining *Lantana camara* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in-vitro*

% Mortality of RKN J2s treated with varying concentrations of *Lantana* leaf extract combined with *C. nigrum*						
	Experiment I			**Experiment II**		
Treatment	**24 Hours**	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**
25% LE + *C. nigrum*	18.50±0.96e	35.0±01.29d	46.00±2.16e	19.50±0.50e	33.50±1.71e	42.00±1.41e
50% LE + *C. nigrum*	32.00±1.41d	41.50±0.96c	53.00±1.29d	33.00±1.29d	45.00±1.26d	62.50±1.71d
75% LE + *C. nigrum*	45.00±1.29c	63.00±2.08b	69.00±1.29c	44.50±1.71c	57.00±1.29c	71.00±1.29c
100% LE + *C. nigrum*	52.00±4.14b	67.00±0.58a	81.00±1.29b	51.00±1.29b	66.00±1.41b	83.00±1.29b
1×10^6 *C. nigrum*	53.00±1.29a	67.50±3.50a	88.00±2.08a	53.50±2.50a	67.00±0.82a	89.00±2.38a
Control (distilled water)	0.00±0.00f	0.00±0.00e	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00f
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

The numbers depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different.

The results indicate that duration of exposure to concentrations of *Lantana* leaf extract combined with *C. nigrum* influenced mortality of RKN J2s with 72 hour period producing the highest mortality.

In both experiments, there was a general trend of increasing mortality of J2s with increasing *Lantana* leaf extract concentrations combined with *C. nigrum* and vice versa (Figure 4.11)

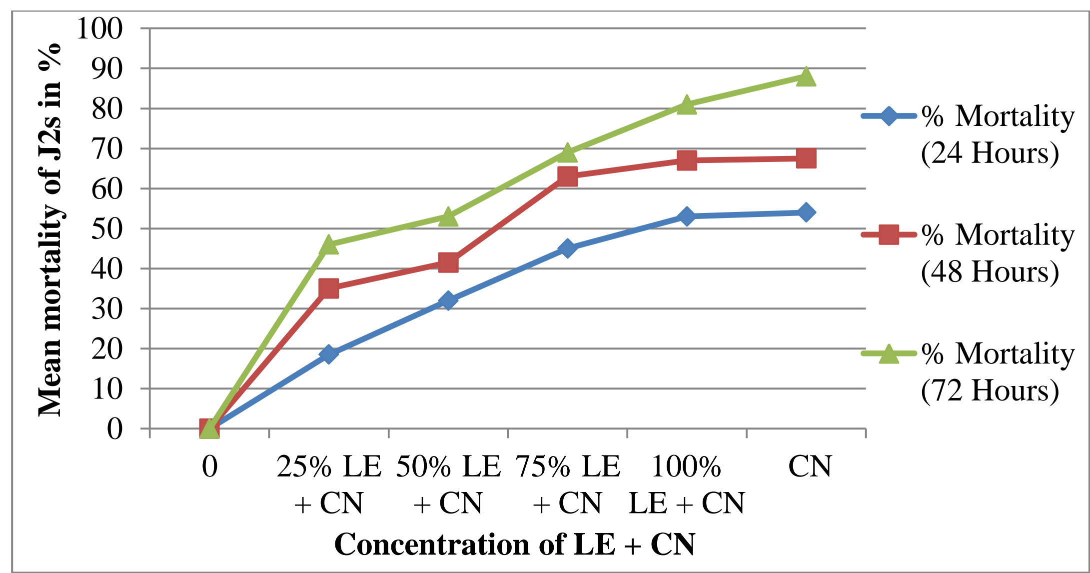


Figure 4:11: Effect of varying concentration of *Lantana* leaf extract combined with *C. nigrum* on mortality of J2s *in vitro*

On further analysis, the time of exposure and mortality of J2s were found to have a correlation that was significantly positive (r=0.94, at P≤0.05) with concentration in *in vitro* tests (Figure 4.12) and the same trend was observed in *in-vitro* test II.

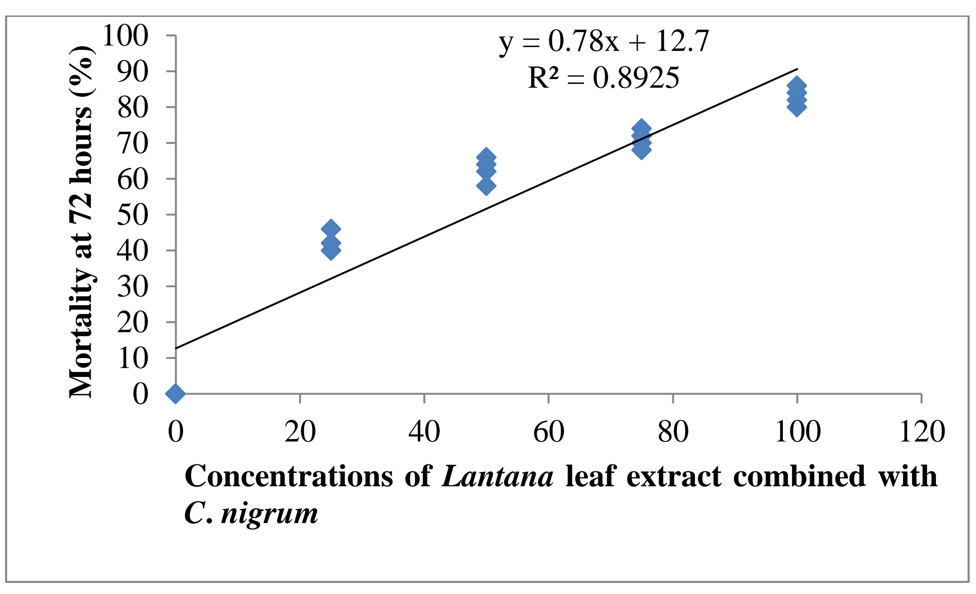


Figure 4:12: Relationship between the time of exposure to various concentrations of *Lantana* leaf extract combined with *C. nigrum* in *in vitro* test

4.4.3 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime against RKNs on tree tomato in the greenhouse tests

i) Effect on population of J2s in soil and roots

All treatments except the control statistically (P≤0.05) lowered the soil J2 populations relative to untreated (positive) control which increased (Tables 4.16 and 4.17). The endophytic *C. nigrum* local isolate + *Lantana* extract + Velum® Prime treatment had the highest significant (P≤0.05) J2 population reduction in 200cc of dry soil although not significantly different in comparison to commercial *T. asperellum* + *Lantana* extract + Velum® Prime treatment. This was followed by Velum® and *Lantana* extract treatments whose J2 reductions were not significant (P≥0.05) between them. The trend in experiment I was similar to the one in experiment II (Tables 4.16 and 4.17).

In roots, the treatments significantly lowered the J2s relative to the un-treated control. The *C. nigrum* isolate + *Lantana* extract + Velum® Prime treatment had the highest J2 reduction in 5g of roots compared to other treatments although not statistically different from commercial *T. asperellum* + *Lantana* extract + Velum® treatment (Tables 4.16 and 4.17). This was followed by Velum® and *Lantana* extract treatments. The *C. nigrum* + *Lantana* extract and *T. asperellum* + *Lantana* extract treatments had a significant difference in terms of J2 reduction in roots relative to control. The least significant J2s population reduction in roots was recorded in biocontrol fungi standalone treatments compared to positive control.

ii) Effect on RKN reproduction factor

The treatments had significant ($P \leq 0.05$) reduction in the RKN reproduction (Rf) in comparison to the control (Tables 4.16 and 4.17). The *C. nigrum* + *Lantana* extract + Velum® Prime and *T. asperellum* + *Lantana* extract + Velum® Prime treated plants significantly ($P \leq 0.05$) had higher reduction of RKN reproduction factor in comparison to other treatments followed by Velum® Prime and *Lantana* extract treatments (Tables 4.16 and 4.17). The treatment with *C. nigrum* + *Lantana* extract treatment did not statistically differ ($P \geq 0.05$) with that of *T. asperellum* + *Lantana* extract in terms of reproduction factor reduction. The least significant reproduction factor reduction was observed in *C. nigrum* and *T. asperellum* standalone treatments relative to positive control.

Table 4.16: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on RKN J2 populations and reproduction factor on tree tomato in the greenhouse test I (March- June 2023)

Treatment	J2s/200cc dry soil	J2s/5g dry roots	Rf
Colletotrichum nigrum + RKN J2s	601.25 ± 27.57bc	229.25 ± 0.75b	0.30 ± 0.01b
Trichoderma asperellum + RKN J2s	608.75 ± 15.46b	235.75 ± 0.48b	0.30 ± 0.01b
Velum® Prime + RKN J2s	347.50 ± 17.50de	137.50 ± 14.51de	0.17 ± 0.01cd
Lantana extract (L.E) + RKN J2s	401.75 ± 2.02de	171.25 ± 1.25cd	0.20 ± 0.00bcd
C. nigrum + L.E + Velum® Prime + RKN J2s	271.25 ± 8.99e	102.50 ± 5.20e	0.14 ± 0.00d
T. asperellum + L.E + Velum® Prime + RKN J2s	280.00 ± 3.54e	107.50 ± 13.15e	0.14 ± 0.00d
C. nigrum + L.E + RKN J2s	545.00 ± 22.73bcd	205.00 ± 7.91bc	0.27 ± 0.01bc
T. asperellum + L.E + RKN J2s	553.75 ± 17.96bc	207.50 ± 6.61bc	0.28 ± 0.01bc
Positive control (RKN J2s alone)	3877.50 ± 128.35a	483.75 ± 14.34b	1.94 ± 0.06a
Negative control (untreated and uninoculated)	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00e
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The ratio of final (Pf) to initial (Pi) nematode populations constituted nematode reproduction factor (**Rf**); **L.E**= *Lantana* extract; Negative control = untreated control without RKN J2s.

Table 4.17: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the greenhouse test II (Aug – Nov 2023)

Treatment	J2s/200cc dry soil	J2s/5g dry roots	Rf
Colletotrichum nigrum + RKN J2s	597.50 ± 17.97b	188.75 ± 17.49b	0.29 ± 0.01b
Trichoderma asperellum + RKN J2s	605.00 ± 7.36b	193.75 ± 12.31b	0.30 ± 0.00b
Velum® Prime + RKN J2s	326.25 ± 37.99d	117.50 ± 4.79cd	0.16 ± 0.02d
Lantana extract (L.E) + RKN J2s	393.75 ± 28.31cd	131.25 ± 2.39cd	0.19 ± 0.01cd
C. nigrum + L.E + Velum® Prime + RKN J2s	278.75 ± 16.50d	101.25 ± 9.66d	0.13 ± 0.02d
T. asperellum + L.E + Velum® Prime + RKN J2s	287.50 ± 15.07d	105.00 ± 7.91d	0.14 ± 0.01d
C. nigrum + L.E + RKN J2s	540.00 ± 6.46bc	153.75 ± 14.63bc	0.27 ± 0.00bc
T. asperellum + L.E + RKN J2s	528.75 ± 7.47bc	156.25 ± 4.27bc	0.26 ± 0.00bc
Positive control (RKN J2s alone)	3795.00 ± 102.69a	466.25 ± 10.08a	1.90 ± 0.05a
Negative control (untreated and uninoculated)	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00e
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The ratio of final (Pf) to initial (Pi) nematode populations constituted nematode reproduction factor (**Rf**); **L.E**= *Lantana* extract; Negative control = untreated control without RKN J2s.

A regression analysis revealed a positive linear relationship between nematode reproduction factor (Rf) and RKN J2 populations. A further investigation indicated a relationship that was significant (r=0.10, at P=0.05) among the soil's Rf and population of J2s (Figure 4.13).

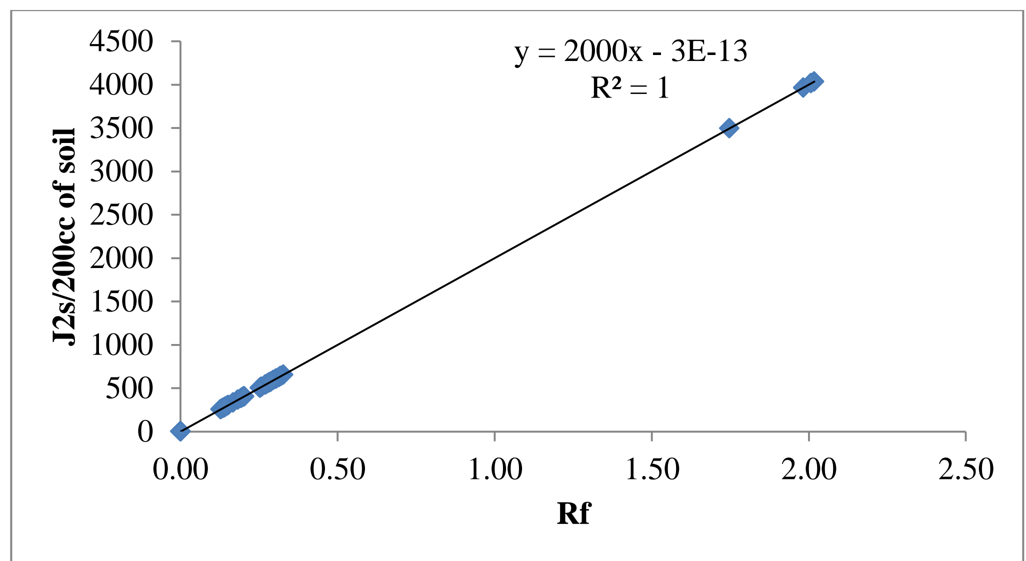


Figure 4:13: Regression analysis between nematode reproduction factor and population of J2s in the soil (Greenhouse test)

iii) Effect on indices of gall and egg masses

In relation to the positive control, other treatments had a significant (P≤0.05) reduction on the indices of galls and egg masses on roots (Table 4.18). *Colletotrichum nigrum* + *Lantana* extract + Velum® Prime had statistically (P≤0.05) higher reduction of indices of gall index d egg masses than other treatments (Plate 4.5). This was followed by *T. asperellum* + *Lantana* extract; Velum® Prime and *Lantana* extract treatments. The least indices on galls and egg masses reductions were recorded in *C. nigrum* and *T. asperellum* standalone treatments (Table 4.18).

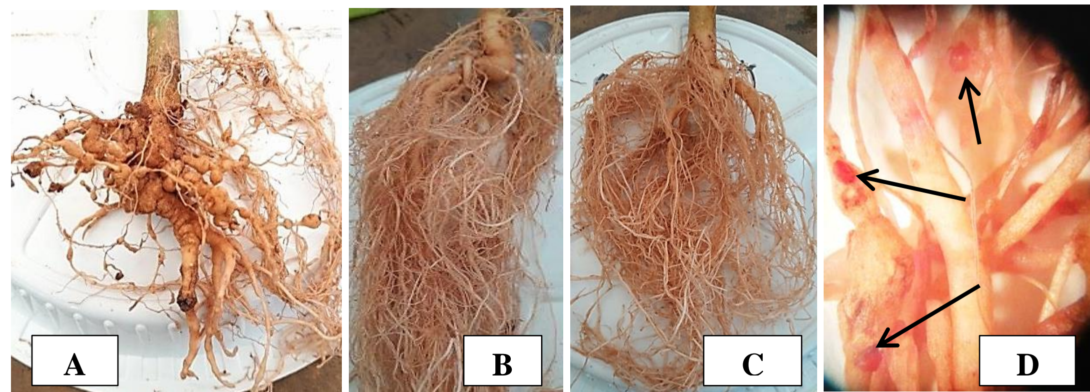


Plate 4.5: Effect of root-knot nematode infestation on indices of galling and egg masses on roots of tree tomato in the greenhouse test.
A: positive control, gall index =5; **B:** Negative control, gall index = 0; **C:** Roots from plants treated with Velum® Prime in combination with *Lantana* extract and *C. nigrum* treatment and **D:** RKN egg masses pointed at by the arrows. **Source:** Own photos, 2023

Table 4.18: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on indices of galls and egg masses on tree tomato in the greenhouse tests (March- June, 2023)

	TEST I		TEST II	
Treatment	**Gall index**	**Egg mass index**	**Gall index**	**Egg mass index**
Colletotrichum nigrum + RKN J2s	3.75 ± 0.48ab	3.50 ± 0.29b	3.50 ± 0.29b	3.50 ± 0.29b
Trichoderma asperellum + RKN J2s	3.75 ± 0.48ab	3.50 ± 0.29b	3.50 ± 0.29b	3.50 ± 0.29b
Velum® Prime + RKN J2s	2.25 ± 0.25bcd	2.00 ± 0.00de	2.25 ± 0.25cd	2.00 ± 0.00cde
Lantana extract (L.E) + RKN J2s	2.50 ± 0.29bcd	2.50 ± 0.29cd	2.50 ± 0.29bcd	2.50 ± 0.29bcde
C. nigrum + L.E + Velum® Prime + RKN J2s	1.75 ± 0.48d	1.50 ± 0.29e	1.50 ± 0.29d	1.25 ± 0.25ef
T. asperellum + L.E + Velum® Prime + RKN J2s	2.00 ± 0.00cd	2.00 ± 0.00de	2.00 ± 0.00cd	1.75 ± 0.25de
C. nigrum + L.E + RKN J2s	3.25 ± 0.25bcd	3.00 ± 0.00bc	2.75 ± 0.25bc	3.00 ± 0.041bcd
T. asperellum + L.E + RKN J2s	3.50 ± 0.29abc	3.25 ± 0.25bc	3.00 ± 0.00bc	3.25 ± 0.48bc
Positive control (RKN J2s alone)	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a
Negative control	0.00 ± 0.00e	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00f
P-Value	<.0001	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different; Negative control = untreated control RKN J2s. Gall and egg mass indices were scored on a scale of 0-5 (Quesenberry *et al*., 1989) as indicated in Table 3.1. **L.E** = *Lantana* extract

iv) Effect on plant shoot height

The heights of uninoculated and treated plants were significantly higher ($P \leq 0.05$) than the ones of inoculated plants (Tables 4.19 and 4.20). *Colletotrichum nigrum* + *Lantana* leaf extract + Velum® Prime treated plants produced the highest height of shoot though not significantly different from that of *Trichoderma asperellum* + *Lantana* leaf extract + Velum® Prime and *C. nigrum* + *Lantana* leaf extract treatments (Tables 4.19 and 4.20). This was followed by *C. nigrum* and *T.* treatments. The least height of shoot increase was exhibited on velum® Prime treated plants followed by those treated with *Lantana* leaf extract.

The heights of shoot of nematode-inoculated plants significantly differed ($P \leq 0.05$) among the treatments (Tables 4.19 and 4.20). Nematode-inoculated plants treated with *C. nigrum* + *Lantana* leaf extract + Velum® Prime had the highest height of shoot followed by those treated with *T. asperellum* + *Lantana* leaf extract + Velum® Prime and Velum® Prime in relation to the positive control. The least height of shoot increase was recorded in *C. nigrum* and *T. asperellum* treated plants.

v) Effect on weights of dry root and shoot

The weights for dry root of uninoculated plants were significantly heavier ($P \leq 0.05$) than the ones inoculated and the positive control (Tables 4.19 and 4.20). Uninoculated plants treated with *C. nigrum* + *Lantana* leaf extract + Velum® Prime had the highest dry root weight followed by *T. asperellum* + *Lantana* leaf extract + Velum® Prime and *C. nigrum* + *Lantana* leaf extract treatments in relation to the negative control (Tables 4.19 and 4.20). The nematode-inoculated and treated plants showed significantly heavier ($P \leq 0.05$) weights of dry root in comparison to positive control as

indicated in Tables 4.19 and 4.20). The *C*. *nigrum* + *Lantana* leaf extract + Velum® Prime treated plants had the highest weights of dry root followed by *T*. *asperellum* + *Lantana* extract + Velum® Prime and *C*. *nigrum* + *Lantana* leaf extract treatment compared to positive control.

The weights of dry shoot of uninoculated plants were significantly heavier ($P \leq 0.05$) relative to those inoculated and positive control as shown in Tables 4.19 and 4.20. Uninoculated plants treated with *C*. *nigrum* + *Lantana* leaf extract + Velum® Prime had the highest weight of dry shoot followed by *T*. *asperellum* + *Lantana* leaf extract + Velum® Prime and *C*. *nigrum* + *Lantana* leaf extract treatments in relation to the negative control. The weights of dry shoot of inoculated and treated plants were statistically higher ($P \leq 0.05$) relative to the positive control (Tables 4.19 and 4.20). The *C*. *nigrum* + *Lantana* leaf extract + Velum® Prime treated plants had the highest weights of dry shoot followed by *T*. *asperellum* + *Lantana* leaf extract + velum and *C*. *nigrum* + *Lantana* leaf extract treatments relative to positive control.

Table 4.19: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height and dry weights of tree tomato in the greenhouse test I (March- June, 2023)

Treatment	Shoot height	Dry root weight	Dry shoot weight
Colletotrichum nigrum + RKN J2s	32.75 ± 2.98g	4.54 ± 0.49d	7.90 ± 0.32e
C. nigrum - RKN J2s	58.14 ± 0.31a	11.92 ± 0.12a	14.31 ± 0.02a
Trichoderma asperellum + RKN J2s	32.25 ± 0.92g	4.49 ± 0.38d	7.83 ± 0.24e
T. asperellum - RKN J2s	57.91 ± 0.35a	11.71 ± 0.14a	14.29 ± 0.02a
Velum® Prime + RKN J2s	39.40 ± 0.61e	5.39 ± 0.14cd	9.57 ± 0.49d
Velum® Prime - RKN J2s	46.33 ± 0.53c	9.13 ± 0.62b	11.77 ± 0.31c
Lantana extract (L.E) + RKN J2s	36.23 ± 0.26f	5.14 ± 0.29cd	9.04 ± 0.30d
Lantana extract (L.E) - RKN J2s	48.94 ± 0.48c	10.57 ± 0.50b	12.26 ± 0.01a
C. nigrum + L.E + Velum® Prime + RKN J2s	48.45 ± 0.45c	7.07 ± 0.40b	11.91 ± 0.03c
C. nigrum + L.E + Velum® Prime - RKN J2s	59.07 ± 0.28a	12.09 ± 0.06a	15.89 ± 0.01a
T. asperellum + L.E + Velum® Prime + RKN J2s	43.75 ± 0.48d	6.45 ± 0.27c	11.61 ± 0.36c
T. asperellum + L.E + Velum® Prime - RKN J2s	58.88 ± 0.29a	12.06 ± 0.06a	15.43 ± 0.02a
C. nigrum + L.E + RKN J2s	35.75 ± 1.01f	5.09 ± 0.26cd	8.71 ± 0.39de
C. nigrum + L.E - RKN J2s	58.37 ± 0.27a	12.03 ± 0.07a	14.66 ± 0.03a
T. asperellum + L.E + RKN J2s	34.68 ± 0.91f	4.85 ± 0.44d	8.62 ± 1.56de
T. asperellum + L.E - RKN J2s	54.59 ± 0.21b	11.99 ± 0.11a	14.11 ± 0.05a
Positive control (RKN J2s alone)	25.45 ± 0.84h	3.70 ± 0.33e	4.93 ± 0.35f
Negative control (untreated and uninoculated)	52.63 ± 0.39b	9.22 ± 0.02b	13.28 ± 0.25b
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different; **L.E** = *Lantana* leaf extract; Negative control = untreated control RKN J2s.

Table 4.20: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height and dry weights of tree tomato in the greenhouse test II (August – November, 2023)

Treatment	Soot height	Dry root weight	Dry shoot weight
Colletotrichum nigrum + RKN J2s	30.88 ± 1.86f	4.24 ± 0.29d	7.17 ± 0.10de
C. nigrum - RKN J2s	57.39 ± 0.41a	11.77 ± 0.10a	14.09 ± 0.24ab
Trichoderma asperellum + RKN J2s	29.63 ± 1.70f	3.80 ± 0.06e	7.04 ± 0.10de
T. asperellum - RKN J2s	56.79 ± 0.43a	11.59 ± 0.12a	14.01 ± 0.21ab
Velum® Prime + RKN J2s	38.00 ± 3.75cd	5.43 ± 0.17cd	8.92 ± 0.49d
Velum® Prime - RKN J2s	45.44 ± 0.52c	9.07 ± 1.01b	11.63 ± 1.12b
Lantana extract (L.E) + RKN J2s	35.00 ± 1.57d	5.020 ± .27d	8.71 ± 0.06d
Lantana extract (L.E) - RKN J2s	47.88 ± 0.67c	10.23 ± 2.21ab	12.05 ±1.10 b
C. nigrum + L.E + Velum® Prime + RKN J2s	47.70 ± 0.77c	7.19 ± 0.27c	10.83 ± 0.26c
C. nigrum + L.E + Velum® Prime - RKN J2s	58.69 ± 0.38a	12.03 ± 3.16a	15.34 ± 1.01a
T. asperellum + L.E + Velum® Prime + RKN J2s	45.98 ± 1.04c	6.54 ± 0.26c	9.97 ± 0.04c
T. asperellum + L.E + Velum® Prime - RKN J2s	57.95 ± 0.50a	11.97 ± 0.09a	15.07 ± 1.04a
C. nigrum + L.E + RKN J2s	33.50 ± 2.04e	4.83 ± 0.40d	8.52 ± 0.52d
C. nigrum + L.E - RKN J2s	57.51 ± 0.44a	11.96 ± 0.11a	14.57 ± 0.29ab
T. asperellum + L.E + RKN J2s	33.00 ± 3.95e	4.52 ± 0.21d	8.19 ± 0.06d
T. asperellum + L.E - RKN J2s	55.19 ± 1.23ab	11.90 ± 0.11a	14.15 ± 0.25ab
Positive control (RKN J2s alone)	22.45 ± 1.11g	2.63 ± 0.28f	4.77 ± 0.45e
Negative control (untreated and uninoculated)	54.38 ± 0.66b	9.60 ± 0.49a	12.83 ± 0.04b
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different; **L.E** = *Lantana* leaf extract; Negative control = untreated control without RKN J2s.

4.4.4 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime against RKNs on tree tomato in the field, season I and II at Njabini and Engineer respectively

i) Effect on population of J2s in soil and roots

All treatments reduced the soil final nematode populations (Pf) relative to positive control (untreated control) (Tables 4.21 and 4.22). The local *C. nigrum* + *Lantana* extract + Velum® Prime exhibited significantly ($P \leq 0.05$) highest J2 reduction in 200cc of dry soil followed by commercial *T. asperellum* + *Lantana* extract + Velum® Prime; Velum® Prime ; *Lantana* extract; *C. nigrum* + *Lantana* extract and commercial *T. asperellum* + *Lantana* extract treatments. The local *C. nigrum* and commercial *T. asperellum* standalone treatments had the least final soil nematode reduction relative to positive control.

In roots, the treatments lowered the J2s relative to the positive control. The local *C. nigrum* + *Lantana* extract + Velum® Prime and commercial *T. asperellum* + *Lantana* extract + Velum® treatments statistically ($P \leq 0.05$) reduced the J2s in plant roots though not significantly different from each other (Tables 4.21 and 4.22). This was followed by Velum® Prime; *Lantana* extract; *C. nigrum* + *Lantana* extract and commercial *T. asperellum* + *Lantana* extract treatments. The least J2 reductions in roots were observed in local *C. nigrum* and commercial *T. asperellum* standalone treatments.

ii) Effect on RKN reproduction factor

The nematode reproduction factor (Rf) showed significant ($P \leq 0.05$) reduction by the treatments except the positive control (Tables 4.21 and 4.22). The local *C. nigrum* + *Lantana* extract + Velum® Prime had the highest significant reduction of nematode Rf

followed by commercial *T. asperellum* + *Lantana* extract + velum® Prime; velum® Prime; *Lantana* extract; *C. nigrum* + *Lantana* extract and commercial *T. asperellum* + *Lantana* extract treatments. The least RKN Rf reductions were observed in local *C. nigrum* and commercial *T. asperellum* standalone treatments relative to positive control.

Table 4.21: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the field season I (March- June, 2023)

Treatment	J2s/200cc dry soil	J2s/5g roots	Rf
Colletotrichum nigrum	227.50 ± 10.10bc	61.25 ± 6.25bc	0.74 ± 0.03b
Trichoderma asperellum	241.25 ± 5.57b	65.00 ± 6.77b	0.76 ± 0.02b
Velum® Prime	122.50 ± 8.54def	25.00 ± 9.79de	0.24 ± 0.01c
Lantana extract (L.E)	151.25 ± 14.05de	28.75 ± 9.44cde	0.69 ± 0.07b
C. nigrum + L.E + Velum® Prime	86.25 ± 21.05f	11.25 ± 2.39e	0.21 ± 0.06c
T. asperellum + L.E + Velum® Prime	91.25 ± 25.61ef	13.75 ± 4.27e	0.22 ± 0.06c
C. nigrum + L.E	170.00 ± 2.38cd	48.75 ± 6.25bcd	0.59 ± 0.02b
T. asperellum + L.E	172.50 ± 4.33cd	51.25 ± 5.54bcd	0.59 ± 0.03b
Positive control (RKN J2s alone)	1441.25 ± 8.51a	196.75 ± 9.10a	3.53 ± 0.12a
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The ratio of final (Pf) to initial (Pi) nematode populations constituted nematode reproduction factor (**Rf**); **Positive control** = untreated control with RKN J2s.

Table 4.22: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on J2 populations and RKN reproduction factor on tree tomato in the field season II (August - November, 2023)

Treatment	J2s/200cc dry soil	J2s/5g in roots	Rf
Colletotrichum nigrum	212.50 ± 3.23bc	58.75 ± 6.88b	0.72 ± 0.01b
Trichoderma asperellum	231.25 ± 10.68b	62.50 ± 5.20b	0.75 ± 0.08b
Velum® Prime	118.75 ± 14.63ef	23.75 ± 4.27d	0.30 ± 0.04de
Lantana extract (L.E)	147.50 ± 3.23de	27.50 ± 3.23cd	0.48 ± 0.03cd
C. nigrum + L.E + Velum® Prime	77.50 ± 8.29f	8.75 ± 5.54d	0.15 ± 0.01e
T. asperellum + L.E + Velum® Prime	85.00 ± 12.75f	10.00 ± 2.04d	0.21 ± 0.04e
C. nigrum + L.E	167.50 ± 3.23d	46.25 ± 4.27bc	0.56 ± 0.03bc
T. asperellum + L.E	170.00 ± 7.91cd	50.00 ± 3.53b	0.69 ± 0.04b
Positive control (RKN J2s alone)	1540.00 ± 10.41a	135.00 ± 5.40a	3.06 ± 0.07a
P-Value	<.0001	<.0001	

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different. **J2s** = second stage juveniles of RKNs. The ratio of final (Pf) to initial (Pi) nematode populations constituted nematode reproduction factor (**Rf**); **Positive control** = untreated control with nematodes.

A regression analysis revealed a positive linear relationship between nematode reproduction factor (Rf) and RKN J2 populations. A further analysis showed a strong correlation that was positively significant (r=0.99, at P=0.05) between nematode reproduction factor and populations of J2s in the soil (Figure 4.14).

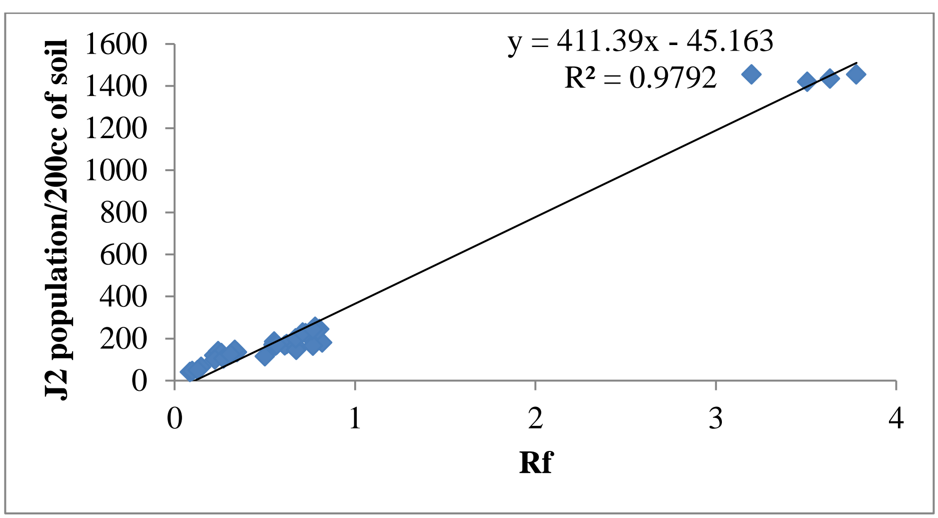


Figure 4:14: Regression analysis between nematode reproduction factor and population of J2s in the soil (Field expt.)

iii) Effect on indices of galls and egg masses

There was existence of statistical differences (P≤0.05) shown by the treatments except control on reduction of indices of galls and egg masses on tree tomato (Table 4.23). The *C. nigrum* + *Lantana* extract + Velum® Prime treated plants had the highest reduction of indices of galls and egg masses followed by commercial *T. asperellum* + *Lantana* extract + Velum® Prime; Velum® Prime; *Lantana* extract; *C. nigrum* + *Lantana* extract and commercial *T. asperellum* + *Lantana* extract treatments. However, the least indices on galls and egg masses were recorded in plants treated with *C. nigrum* and commercial *T. asperellum* standalone treatments relative to positive control.

Table 4.23: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on indices of galling and egg masses on tree tomato in the field (season I - March - June; season II - Aug - Nov. 2023)

	Season I		**Season II**	
Treatment	**Gall indices**	**Egg mass indices**	**Gall indices**	**Egg mass indices**
Colletotrichum nigrum	3.00 ± 0.41abc	2.93 ± 0.22bc	2.75 ± 0.25b	2.74 ± 0.14b
Trichoderma asperellum	3.25 ± 0.63ab	3.05 ± 0.26b	3.15 ± 0.00b	3.00 ± 0.00b
Velum® Prime	1.50 ± 0.20cde	1.38 ± 0.08de	1.25 ± 0.14c	1.31 ± 0.12de
Lantana extract (L.E)	1.50 ± 0.35cde	1.49 ± 0.09de	1.30 ± 0.12c	1.44 ± 0.16cd
C. nigrum + L.E + Velum® Prime	0.75 ± 0.25e	0.56 ± 0.06e	0.75 ± 0.14c	0.50 ± 0.00e
T. asperellum + L.E + Velum® Prime	0.90 ± 0.28de	0.81 ± 0.16e	0.880 ± .24c	0.75 ± 0.18de
C. nigrum + L.E	2.50 ± 0.00bcd	2.00 ± 0.24cd	2.25 ± 0.25b	2.31 ± 0.21bc
T. asperellum + L.E	2.63 ± 0.29bc	2.13 ± 0.22bcd	2.63 ± 0.24b	2.56 ± 0.41b
Positive control (RKN J2s alone)	4.25 ± 0.25a	4.13 ± 0.32a	4.00 ± 0.20a	4.00 ± 0.00a
P-Value	<.0001	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at P≤0.05, the means in a column followed by different letter(s) are statistically different; Negative control = untreated control without RKN J2s. Gall and egg mass indices were scored on a scale of 0-5 (Quesenberry *et al*., 1989) as indicated in Table 3.1. **L.E** = *Lantana* extract.

iv) Effect on plant shoot height

The heights of treated plant shoots were higher relative to positive control (untreated plants) as shown in Tables 4.24 and 4.25. The *C. nigrum* + *Lantana* extract + Velum® Prime and *T. asperellum* + *Lantana* extract + Velum® Prime treated plant shoots exhibited significantly (P≤0.05) higher height relative to other treatments. This was followed by Velum® Prime; *Lantana* extract; *C. nigrum* + *Lantana* extract and *T. asperellum* + *Lantana* extract treatments. The *C. nigrum* and *T. asperellum* treated plants had lower height of shoot relative to positive control.

v) Effect on weights of dry root and shoot

There was an increase in weights of dry shoot and root by the treatments except the positive control (Tables 4.24 and 4.25). The plants treated with *C. nigrum* + *Lantana* extract + Velum® Prime and those treated with *T.* a*sperellum* + *Lantana* extract + Velum® Prime produced the highest significant (P≤0.05) weights of dry shoot and root relative to positive control followed by Velum® Prime and *Lantana* leaf extract treatments (Tables 4.24 and 4.25). The *C. nigrum* and *T. asperellum* treated plants recorded lower weights of dry shoot and root but more than the positive control.

Table 4.24: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height (SH); dry root weights (DRW) and dry shoot weight (DSW) of tree tomato in the field season I (March- June, 2023)

Treatment	SH	DRW	DSW
Colletotrichum nigrum	31.93 ± 1.90cd	8.10 ± 0.51bcd	25.95 ± 2.76de
Trichoderma asperellum	31.60 ± 1.70cd	8.01 ± 1.10bcd	24.12 ± 3.10de
Velum® Prime	38.45 ± 1.40ab	10.11 ± 1.54ab	30.71 ± 1.29bc
Lantana extract (L.E)	36.34 ± 0.64ab	9.70 ± 0.41abc	29.14 ± 5.50bc
C. nigrum + L.E + Velum®	43.24 ± 1.62a	10.82 ± 2.14a	34.18 ± 1.21a
T. asperellum + L.E + Velum® Prime	42.72 ± 2.17a	10.26 ± 1.01a	33.30 ± 2.35ab
C. nigrum + L.E	34.78 ± 2.77bcd	9.59 ± 0.78abc	28.93 ± 3.28bc
T. asperellum + L.E	33.71 ± 1.52bcd	8.93 ± 1.22bc	26.56 ± 1.52de
Positive control (RKN J2s alone)	29.61 ± 3.03d	6.84 ± 0.84d	20.13 ± 3.89f
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different; **Positive control** = untreated control with RKN J2s.

Table 4.25: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana* leaf extract and Velum® Prime on shoot height (SH); dry root weights (DRW) and dry shoot weight (DSW) of tree tomato in the field season II (August - November, 2023)

Treatment	SH	DRW	DSW
Colletotrichum nigrum	32.21 ± 1.08cd	7.99 ± 0.27bcd	25.21 ± 0.27de
Trichoderma asperellum	31.16 ± 1.13cd	7.77 ± 0.25cd	23.96 ± 0.69ef
Velum® Prime	37.67 ± 1.09ab	9.64 ± 0.20ab	30.20 ± 0.54bc
Lantana extract (L.E)	36.75 ± 0.94ab	9.02 ± 0.48abc	28.96 ± 0.26c
C. nigrum + L.E + Velum® Prime	41.25 ± 1.11a	10.08 ± 0.67a	33.82 ± 0.71a
T. asperellum + L.E + Velum® Prime	40.08 ± 0.89a	9.97 ± 0.28a	32.84 ± 0.59ab
C. nigrum + L.E	34.16 ± 0.40bc	8.56 ± 0.15abc	28.31 ± 0.29cd
T. asperellum + L.E	33.22 ± 0.87bcd	8.00 ± 0.44bc	27.33 ± 0.24cd
Positive control (RKN J2s alone)	28.98 ± 0.74d	6.38 ± 0.06d	21.77 ± 1.56f
P-Value	<.0001	<.0001	<.0001

The values depict means ± SE of four replicates. With Tukey's Honestly significant difference (HSD) test at $P \leq 0.05$, the means in a column followed by different letter(s) are statistically different; **Positive control** = untreated control with RKN J2s.

CHAPTER FIVE: DISCUSSION

5.1 Efficacy of endophytic fungal isolates on mortality of RKN J2s

Most of the endophytic fungal isolates tested for mortality of RKN J2s showed significant differences in their activity after 24, 48 and 72 hours respectively. Twenty isolates out of the forty four achieved J2 mortality rates of 50 % and above at the end of 72 hours. Among these, *Colletotrichum nigrum*, *Aspergillus ustus*, *Fusarium solani* and *F. oxysporum* caused more than 60 % mortality of J2 after 72 hours with *C. nigrum* achieving more than 80 %. These results corroborate other studies on tomatoes where *Colletotrichum* spp., *Fusarium* spp. and *Aspergillus* spp. were established to be effective against J2s *in vitro* (Dorcus *et al*., 2010; Kariuki *et al*., 2021). Naz *et al*. (2021) reported significant mortality of *Meloidogyne incognita* J2s using fungal filtrates of *Aspergillus flavus*, *Penicillium chrysogenum* and *Pochonia chlamydosporia* at 100 % concentration with 72 hours of exposure. Endophytic *Acremonium sclerotigenum* isolated from tomato root galls caused by *M. incognita* significantly increased mortality of J2s up to 95.5 % and inhibited egg hatching of *M. incognita in-vitro* by up to 43 % (Yao *et al*., 2023). Ganeshan *et al*. (2021) reported five endophytic fungal filtrates that showed 100 % inhibition of egg hatching and over 80 % mortality of *M. incognita* J2s after 72 hours exposure.

Endophytic fungi act against nematodes by parasitism, paralysis, antibiosis, and by production of lytic enzymes (Poveda *et al*., 2020). Many endophytic fungi have also been reported to parasitize nematode J2s or use their hyphae to trap nematodes or produce nematicidal compounds (Kumar and Dara, 2021; Grabka *et al*., 2022; Kumar *et al*., 2023). *Colletotrichum* spp. is known to produce tyrosol (Talukdar *et al*., 2021) and

colletotricholides A and B while *Fusarium oxysporum* is known to produce 4-hydroxybenzoic acid (Meyer *et al*., 2020) and other nematotoxic substances against nematode J2s (Bogner *et al*., 2017). The observed mortality of J2s in this study could have been caused by endophytic fungi producing toxic substances, lytic enzymes and through parasitim. The 0.25 and 0.5% mortalities of J2s found in the control may have been due to lack of oxygen in the suspension or due to shock that could have come from the extraction process especially during blending.

Colletotrichum sp. has been reported as an endophyte but their potential as biocontrol agent had not been clearly elucidated (Guevara-Suarez, *et al*., 2020). This study reveals that endophytic *Colletotrichum nigrum* has potential use as a nematode antagonist.

5.2 Endophytic fungi of tree tomato from Nyandarua County

Several endophytic fungi were extracted from healthy roots of tree tomato and eggs of RKN from Nyandarua County in this study. The endophytic fungi comprised *Colletotrichum* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillim* spp., *Didymella* spp., *Periconia* spp., *Purpureocillim* spp., *Rhizoctonia* spp. and *Microsphaeropsis* species. Dong *et al*. (2021) observed that endophytic fungal diversity was highest in roots as compared to other parts because roots are in the soil environment which is rich in microbes and root surface area is larger than other parts. Roots are the major entry points of soil-borne fungi into the host plants.

This study identified *Fusarium* spp. as the most abundant in roots from the sampled farms in Nyandarua County (Figure 4.1). *Fusarium* spp. has also been isolated

at higher frequencies from farms in other parts of Kenya (Kibunja, 2015; Kariuki *et al*., 2021). This reveals that endophytic *Fusarium* spp. is abundant and widespread in tree tomato growing areas. Many fungal endophytes including *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Phoma* spp., *Periconia* spp., *Microsphaeropsis* spp., *Talaromyces* spp., and *Trichoderma* spp. were isolated from roots of rice in irrigated and upland ecosystems in Kenya (Pili, 2016). Kariuki *et al*. (2021) also extracted *Trichoderma* spp., *Penicillium* spp., *Purpureocillium* spp. and *Fusarium* species from roots of tomato and *Meloidogyne* eggs in Mwea, Kirinyaga County in Kenya. Similar study carried out in Bangladesh found that *Fusarium* spp., *Aspergillus* spp. and *Penicillium*s spp. were associated with RKN eggs and females (Aminuzzaman *et al*., 2018). Other studies have also isolated *Aspergillus* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Phyllostica* spp., and *Alternaria* spp., with the highest frequency on high-risk imported ornamental plants (Gioia *et al*., 2020). *Colletotrichum* endophytes have also been extracted from roots, stems and leaves of *Nothapodytes pittosporoides* plants in China (Zhou *et al*., 2019) and from stems of *Artemisia annua* (Liu *et al*., 2000). Many endophytic fungi with antagonistic potential against RKNs have been isolated on cotton (Zhou *et al*., 2020). *Colletotrichum* spp occur in many plants as endophytes, pathogens or as saprobes (Guevara-Suarez *et al*., 2022).

Morphological characteristics were used in identification of endophytic fungal isolates into various species. Colony colour, presence or absence of microconidia, macroconidia and chlamydospores and colony characteristics were used to differentiate between species. These features have been used to identify various fungi (Kibunja, 2015).

However, the morphological features alone cannot be used to conclusively identify the fungal endophytes. Therefore, ITS region of the rDNA was used to separate the taxa further to the level of species complex. From the twenty isolates that were effective against RKN J2s, eighteen isolates were conclusively identified through molecular DNA sequencing. From the results of phylogenetic study, it was shown that ITS sequences can be effectively used to identify and show relationships among the endophytic fungi (Guevara-Suarez, *et al*., 2020). Molecular identification on the sequences in BLASTn database was determined by percentage similarity (93 - 100 %) and query cover (96 – 100 %). The genebank accession codes for the isolates from this research study have been deposited at the NCBI database. Clade I had 17 isolates belonging to phyllum Ascomycota while Clade II had only one isolate (*Rhizoctonia solani*) that belong to Basidiomycota. The difference in phylla abundance could be due to their different ways of reproduction and spore development. Ascomycetes have highly explosive mechanisms of dispersal of endogenous sexual spores which leads to wider ranges of distribution as compared to exogenous asexual spores of Basidiomycetes. All *Aspergillus* sp. and *Penicillium* sp. were clustered together. These two species belong to the same class separated with 99 % bootstrap value which means that they may have diverged recently. Isolates N31, N14, N33 and N29 affiliated with *Periconia* sp., *Microsphaeropsis arudinis* and *Didymella bellidis*, respectively, were more diverged from the remaining fungal isolates in clade I. This suggests that, as indicated by a higher C/T occurrence, they may have recently diverged as a result of a faster frequency of substitution (transitional) that has led to variance in the DNA.

5.3 Compatibility of Velum® prime with endophytic *Colletotrichum nigrum* and commercial *Trichoderma asperellum* against RKNs on tree tomato

Velum® Prime (ai = fluopyram 500g/L) did not inhibit the colony growths of *C. nigrum* and *T. asperellum* on PDA media. This showed that the two biocontrol fungi are compatible with Velum® Prime. Kibunja.(2015) tested the compatibility of Mocap (active ingredient: ethoprophos= organophosphate) and *Trichoderma* spp. and reported that Mocap had no significant effect on *Trichoderma* spp. This could be explained that Velum® Prime being systemic non-fumigant is relatively less toxic to soil microflora (Wada and Toyota, 2008). Non-fumigants have little effect on organisms that lack the nervous system since they act as cholinesterase inhibitors in the neurons (Bhattacharjee and Dey, 2014).

The reduction in plant disease parameters could be due to production of nematotoxic secondary metabolites (flavonoids, terpenoids, trichodermin, fusaric acid, colletonoic acid among others) and direct parasitism by the biocontrol fungi. Other studies have shown that endophytic fungi used for biocontrol produce toxic metabolites against RKNs (Meyer *et* al., 2020; Kumar *et al*., 2023; Yao *et al*., 2023). Velum® Prime is known to act against soil pathogens and enhance plant health (Chen *et al*., 2020). This could explain the reduced disease parameters by the combined application of biocontrol fungi with Velum®. Other studies have highlighted the nematicidal effect of Velum® Prime (Dahlin, *et al*., 2019). *Colletotrichum nigrum* could have worked synergistically with Velum® against the RKN J2s resulting into decreased disease parameters. The results from this work are in agreement with Dahlin, *et al*. (2019) who noted that combining *Purpureocilliun lilacinum* (strain PL251) with Velum® Prime (active

ingredient = fluopyram) reduced J2 populations of *M. incognita* by 68 % and gall index to 1.8 as compared to control (3.8). The same treatment also enhanced yield of tomatoes under greenhouse tests.

The biocontrol fungi enhanced growth parameters of tree tomato when applied alone and in combination with Velum® Prime (ai = fluopyram 500g/L) in the greenhouse tests. As standalone applications, these biocontrol fungi (*C. nigrum* and *T. asperellum*) could be possessing plant growth promoting abilities. This could explain the increased shoot heights and dry weights of root and shoot of tree tomato plants. Some fungi like *Trichoderma* spp. are known to act as biofertilizers which improves plant growth (Kubheka and Ziena, 2022) and have been commercialized in Kenya. *Trichoderma* spp. have been used to produce volatile chemicals (alkaloids, colletonoic acid, esters, alcohols, ketones etc) against soil pathogens, solubilizes phosphates to make them available for plant absorption in acidic soils and also enhances uptake of micro and macro- nutrients by plants (Kubheka and Ziena, 2022). The endophytic fungi are also known to confer protection to plants against other pathogens (Silva Santos *et al*., 2022) and this could explain the reduced plant disease parameters upon their application. Another research study showed that endophytic *Colletotrichum tofieldiae* promoted plant growth of maize and tomato *in-vitro* resulting into higher shoot heights and weights (Conzalez-Diaz Sandra, *et al*,. 2020). Silva Santos *et al*. (2022) also found out that inoculating tomato plants with *Colletotrichum siamense* increased plant biomass. As combined treatments (Biocontrol fungi + Velum® Prime (ai = fluopyram 500g/L) were able to enhance plant growth parameters (shoot height and dry weights)

and reduced plant disease parameters (nematode populations, egg masses and gall indices and reproduction factor).

The findings of this study could be used to promote integrated nematode management using less toxic nematicides and biocontrol agents especially under field conditions where efficacy of BCAs is limited (Koll *et al*, 2011).

5.4 Efficacy of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with *Lantana camara* leaf extract and Velum® Prime against root-knot nematodes on tree tomato

In-vitro test revealed that 25, 50, 75 and 100 % concentrations of *Lantana* leaf extract had significant mortality of J2s at 24, 48 and 72 hours of exposure. The 100 % *Lantana* leaf extract concentration was the most effective against the J2s. Increase in the time of exposure directly increased the rate of mortality of J2s. These findings agree with Feysia *et al*. (2016) who reported 96 % mortality of J2s at 72 hours of exposure to *Lantana* leaf extracts. Khan *et al*. (2019) on evaluation of some botanicals (*Coccinia grandis*, *Commelina benghalensis*, *Leucas cephalotes*, *Phyllanthus amarus* and *Trianthema portulacastrum*) against RKN, *Meloidogyne incognita* on carrot found out that 5000 ppm concentration of the botanical extracts had the highest mortality of J2s compared to 1000 ppm concentration. Research by Bordoloi *et al*. (2021) reported 91.6 % mortality of J2s *in-vitro* in 100 % concentration after 96 hours when working on mechanism of *L. camar*a leaf extract in the management of *M*. *incognita* on tomato. Plant extracts contain nematicidal chemical compounds (Chitwood, 2002; Bordoloi *et*

al., 2021). It has been shown that plant extracts contain bioactive compounds such as organic acids, phenolics, alkaloids, terpinoids and terpenes, coumarins and secondary metabolites that possess nematicidal properties (Shaukat *et al*., 2002; Bordoloi *et al.*, 2021). *Lantana camara* has been shown to release camaric acid, camarinic acid, Lantanolic acid, Linaroside, Oleanoic acid, Lantadene A and B, betulinic acid, Lancamarolide and 11α-hydroxy-3-oxours-12-en-28-oic acid that have nematicidal properties (Begum *et al*., 2015; Gebreyohannes *et al*., 2023). However, Bordoloi *et al*. (2021) reported that lower concentrations (25-50 %) of *Lantana camara* extracts stimulated plant growth on tomato while higher concentrations of 75-100 % inhibited tomato plant growth. The author further noted that peroxidase; polyphenoloxidase and total phenol were highest in 100 % concentrations and lowest in 25 % and 50 % concentrations. This explains the high mortality of J2s in 100 % concentrations. From these results, it is evident that *Lantana* leaf extract has nematicidal effects and this could be due to the presence of nematotoxins of phytochemicals that are soluble in water. Use of plant extracts is an economical and safe method of controlling RKNs. Shaukat *et al*. (2002) showed that plant extracts that contain alkaloids and flavonoids have orvicidal and larvicidal properties on RKN eggs. The reason for *Lantana* leaf extract having nematicidal activity could also be attributed to lipophilic properties of oxygenated compounds which dissolve cytoplasmic membranes of nematodes thus interfering with enzyme protein structure. Plant extracts suppress acetylcholine esterase enzyme activity. These mechanisms could be responsible for mortality of nematode J2s in this research.

The combination of different concentrations of *Lantana* leaf extract with *C. nigrum* significantly ($P \leq 0.05$) killed J2s although lower than when applied as standalone treatments. The reduced mortality of J2s in the combination of *Lantana* leaf extract and *C. nigrum* could be due to either both having some level of inhibition to the other. The length of time of exposure was directly proportional to the mortality of J2s. The results from this work are in agreement with Feysia *et al*. (2016) who reported that the combination of *Lantana* extracts with *Trichoderma harzianum* had 66 % mortality of J2s compared to *Lantana* alone which had 96 %. In the same findings, Feysia *et al*. (2016) found that all botanicals in combination with *T. harzianum* had lower mortality of J2s as compared to when applied alone after 24, 48 and 72 hours. This agrees with the research findings of this study where *Lantana* extracts applied singly significantly reduced J2 populations than when combined with endophytic *C. nigrum*.

Endophytic fungi reduce attack of nematodes to plants by parasitism, paralysis, and antibiosis, production of lytic enzymes and through space competition. They also induce plant defenses like activating systemic acquired resistance (SAR), induced systemic resistance (ISR) and strigolactones production (Poveda *et al*., 2020). Many endophytic fungi have been used in the management of RKNs with different mechanisms of antagonism (Forghan and Hajihassan, 2020).

In this study, the biocontrol fungi (*C. nigrum* and *T. asperellum*) in combination with *Lantana camara* extract and Velum® Prime reduced the J2s population significantly ($P \leq 0.05$), nematode reproduction, indices of galls and egg masses as well as enhancing plant growth parameters. These research findings are similar with those of Akram *et al*. (2020) studying the integrated approach of managing root-knot nematodes,

Meloidogyne graminicola Golden and Birchfield parasitizing on wheat. The author found out that a combined treatment of Cartap (chemical), *Trichoderma harzianum* and neem extracts was most effective in inhibiting egg hatching, increased shoot height and root weight. Similar results were reported where biocontrol fungi as standalone treatments showed slightly reduced efficacy as compared to the chemical nematicides and therefore noted that repeated application is necessary to achieve the desired and sustainable RKN control (Ding *et al*., 2020). El-Ashry *et al*. (2021) working on integrated management of *M*. *incognita* on tomatoes using combinations of biocontrol agents (abamectin, *Purpureocillium lilacinum*, rhizobacteria and botanical) compared with nematicide significantly reduced nematode populations, reduced galling and egg mass indices. Abd-Elgawad. (2020) noted that integrated nematode management cannot only involve compatible RKN strategies but can also utilize different BCA components in combination to friendly chemical nematicides. In his study, the number of potato tubers and tuber weight per plant increased significantly when *Pochonia chlamydosporia*, *Pseudomonas fluorescens* and *Trichoderma viridae* were combined with nematicide carbofuran than either using these BCAs or carbofuran alone. The combined treatment of the BCAs significantly reduced J2 population and eggs (Abd-Elgawad, 2020).

In this study, *C*. *nigrum* and *T*. *asperellum* singly or in combination with *Lantana* leaf extract and Velum® Prime statistically (P≤0.05) reduced RKN disease parameters and improved tree tomato parameters of growth. Other studies have documented the potential of endophytic fungi as biocontrol agents against RKNs (Ganeshan *et al*., 2021; Naz *et al*., 2021; Kumar *et al*., 2023; Yao *et al*., 2023). The

protective and plant growth promoting abilities of endophytic *Colletotrichum* spp. has not been elucidated on tree tomato. However, tomato plants colonized by endophytic *Colletotrichum* spp. showed growth promotion effects in the greenhouse and in the field (Diaz-Gonzalez *et al*., 2020). In their research work, the authors noted that 7 days after inoculation with *Colletotrichum tofieldiae*, tomato seedlings significantly had increased shoot and root length and higher fresh weights relative to untreated control. Further, there was significant increase in the number of buds (64 %), open flowers, (54 %) and dry weight of 2 % that was higher than control. Other research findings were documented on the effects of *Colletotrichum* on different plants such as *Phaseolus vulgaris* (Oliveira *et al*., 2020) and *Arabidopsis thaliana* (Hiruma *et al*., 2016). In other research findings, tomato plants inoculated with *Colletotrichum siamense* (strains JB, 224.g1 and JB, 252.g1) and *Diaporthe masirevicii* (JB, 270) extracted from Mexican shrimp plant (*Justicia brandegeana*) increased the plant biomass and these strains were also positive in the solubilization of phosphate on soil medium and therefore making phosphorous available to plants (Silva Santos *et al*., 2022). This could be the reason for the increased shoot heights and dry weights of tree tomato plants treated with *Colletotrichum* in this study. Marra *et al*. (2012) noted that micro-organisms can produce low molecular weight organic acids that solubilize precipitated forms of phosphorous such as iron and aluminium phosphates in acidic soils and calcium phosphate in basic soils.

Endophytic fungi produce plant hormones such as gibberellins and cytokinins which promote plant growth and enhance plant defense (Skider and Vestergard, 2020). *Colletotrichum* spp. are known for production of plant hormones such as indole-3-acetic

acid which have promoting abilities for plant growth. Both antibiosis and plant growth promoting abilities by *Colletotrichum* enhances plant adaptation and resistance to pathogens by the host plants (Lu *et al*., 2000; Zhou *et al*., 2000). *Colletotrichum gloeosporioides* from *Artemisia mongolica* produces colletotric acid with antibacterial activities (Zhou *et al*., 2000) while *Colletotrichum gloeosporioides*/ XL1200 produces colletotricholides A and B (Zhao *et al*., 2020). In other studies Kiriga *et al*. (2018) working on the effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *M*. *javanica* on commercial pineapple farming reported that *Trichoderma asperellum* effectively reduced galling and egg mass indices by over 82 and 88 %, respectively, and increased root fresh weights by 91 %. In pot experiments, two endophytic fungal culture filtrates of BF7 and BF28 isolates were found to be significantly effective against *M*. *incognita* on bananas by significantly reducing soil and root J2 populations, reducing indices of galling and egg masses on bananas as relative to untreated control (Ganeshan *et al*., 2021).

The idea of integrated nematode management (INM) is gaining attraction in the management of RKNs compared to standalone strategies. Thakur *et al*. (2020) found out that integration of metam sodium with neem cake and *Purpureocillium lilacinum* significantly reduced J2 populations in 200cc of soil as compared to control. The same treatment reduced galls and egg masses and improved yield, root weight and shoot height of cucumber compared to control. Similar results were documented by Dahlin *et al*. (2019) who noted that combining Velum® Prime (ai = fluopyrum) with *Purpureocillium lilacinum* strain 251 (PL 251) enhanced control of *M*. *incognita* and enhanced tomato yield compared to when applied alone in the greenhouse trials.

Velum® Prime protects plants from RKNs by reducing their populations in the soil and protecting plants from nematode penetration. Velum® Prime which is an organochlorine compound is a succinate dehydrogenase inhibitor (SDHI) (Beeman and Tylka, 2018; Chen *et al*., 2020; Touray *et al*., 2020). It selectively inhibits complex II of the mitochondria respiratory chain thereby causing rapid energy depletion in the cells of nematodes which finally kills them. Velum® Prime has excellent quick knock-down effects on nematodes at low concentrations (Chen *et al*., 2020).

Integration of BCAs with compatible nematicides and *Lantana* leaf extract can be used to leverage on their synergistic effects against RKNs to improve plant health and production.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) Twenty isolates of endophytic fungi caused significant mortality (≥ 50 %) of RKN J2s *in vitro* and hence have a potential application as nematode antagonists. Among the isolates, *Colletotrichum nigrum* caused the highest mortality of RKN J2s.

ii) This study shows that roots of tree tomato are a host to diverse community of endophytic fungi. There were forty four endophytic fungi from various genera isolated from roots of tree tomato.

iii) Velum® Prime SC 500 (ai= fluopyram 500g/L) was compatible with endophytic *Colletotrichum nigrum*. Their co-application significantly reduced second stage juveniles of root-knot nematode populations in the soil and roots of tree tomato and therefore have potential use in integrated nematode management especially under field conditions where efficacy of BCAs is limited.

iv) Endophytic *C. nigrum* combined with *Lantana* leaf extract and Velum® Prime was significantly effective against RKNs on tree tomato. Their co-application effectively reduced RKN disease parameters on tree tomato and therefore has potential of being incorporated into integrated nematode management strategies.

6.2 Recommendations

i) Non- pathogenic *Colletotrichum nigrum* which was the most effective endophytic fungus against RKN J2s should be considered for commercialization and be made available to farmers to control nematodes. This endophytic fungus can be incorporated into plant propagation establishments to produce healthy tree tomato seedlings.

ii) There is need to engage extension officers to sensitize farmers about RKN damage on tree tomato and promote use of safe strategies of RKN management such as the use of commercial biocontrol products like the proposed *C. nigrum* based product.

iii) Further research should be conducted to:

a) Establish if other endophytic fungal isolates are compatible with Velum® Prime and other similar synthetic nematicides.

b) Characterize all endophytic fungi associated with tree tomato.

c) Determine the mechanisms of action of the endophytic fungi that were effective against RKN J2s.

iv) There is need to identify other types of bio-nematicides from different plants to replace the harmful and expensive chemical nematicides in use. Integration of BCAs with compatible nematicides and *Lantana* leaf extract to leverage on their synergistic effects against RKNs is preferable. Farmers can start using *Lantana* leaf extract against RKNs as it is readily available and easy to make into the leaf extract with minimal instructions for use.

REFERENCES

Abbasi M. W., Ahmed N., Javed N., Zaki M. J. and Shaukat S. S. (2008). Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. *Pakistan Journal of Botany*, 40:2193-2198.

Abbot, W. S. (1925). A method of computing the effectiveness of insecticide. *Journal of economic entomology*. 18(2), 265-267. Htpps://doi.org/10.1093/jee/18.2.265a

Abd-Elgawad, M. M. M. (2020). Biological control agents in the integrated nematode management of potato in Egypt. *Egyptian journal of biological pest control*. Review. 30:121. https//doi.org/10.1186/s41938-020-00325-x.

Adeleke B. S., Ayilara M. S., Akinola S. A. and Babalola O. O. (2022). Biocontrol mechanisms of endophytic fungi. *Egyptian Journal of biological pest control* 32-46. https://doi.org/10.1186/s41938-022-00547-1. Review.

AFA (Agriculture and Food Authority) and Kenya National Bureau of statistics. (2021). www.agricultureauthority.go.ke/ Validated- *Horticulture-Data-Report* 2019-2020, pages 75-76.

Agenbag, M. (2016). Identification and reproduction potential of South African *Meloidogyne species* [PhD Thesis], North West- University.

Akram, S., Khan, S. A., Javed, N. and Ahmed, S. (2020). Integrated management of root-knot nematode *Meloidogyne graminicola* Golden and Birchfield parasitizing on wheat. *Pakistan journal of zoology*, pp 1-11, 2021. https://dx.doi.org/10.1782/journal.Pj/2019041108043B.

Aminuzzaman, F. M., Jahan S. N., Shammi J., Mitu A. I. and Liu X. Z. (2018): Isolation and screening of fungi associated with eggs and females of root-knot

nematodes and their biocontrol potential against *Meloidogyne incognita* in Bangladesh, *Archives of Phytopathology and Plant Protection*, doi: 10.1080/03235408.2018.1472359.

Beeman, A. Q. and Tylka, G. L., (2018). Assessing the effect of ilevo and votivo seed treatments on reproduction, hatching, motility and root penetration of the soybean cyst nematode, *Heterodera glycines*. *Plant disease*. 102, 107-113. https://doi.org/10.1094/PDIS-04-17-0585-RE.

Begum, S., Ayub A., Siddiqui S. B., Fayyaz S. and Kazi F. (2015). Nematicidal triterpinoids from *Lantana camara*. *Chemistry and biodiversity*. Vol. 12 (2015).

Behdani, M., Pooyan, M., & Abbasi, S. (2012). Evaluation of antifungal activity of some medicinal plants essential oils against *Botrytis cinerea*, causal agent of post harvest apple rot, *in vitro*. *International Journal of Agriculture and Crop Sciences*, *4*(14), 1012-1016.

Bhattacharjee, R., and Dey, U. (2014). An overview of fungal and bacterial bio-pesticides to control plant pathogens/diseases. *African Journal of Microbiology Research*, 8(17): 1749–1762.

Bogner, W. C., Kariuki, M. G., Elashrry, A., Sichtermann, G., Buch, A.K., Mishra, B., Thine, M., Grundler, F.M.W. and Schouten, A. (2016). Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. *Mycological Progress.* 15, 1–17.

Bogner, C.W.; Kamdem, R.S.T.; Sichtermann, G.; Matthäus, C.; Hölscher, D.; Popp, J.; Proksch, P.; Grundler, F. M. W., Schouten, A. (2016). Bioactive secondary metabolites with multiple activities from a fungal endophyte. *Microbiology and*

Biotechnology. 2017, 10, 175–188. [CrossRef] [PubMed] DOI:10.1007/s1157-016-1169-9.

Bordoloi, K., Bhawat, B., Baruah., A. M., Neog, P. P. and Karulka U. (2020). Biochemical mechanism of *Lantana camara* leaf extracts in the management of *Meloidogyne incognita* on tomato. *Journal of pharmacognosy* 10 (1): 2828-2834. https//doi.org/22271/phyto.2021.v10.ilan.13789

Bozbuga R, Lilley C. J, Knox J. P, Urwin P. E. (2018). Host-specific signatures of the cell wall changes induced by the plant parasitic nematode, *Meloidogyne incognita*. *Scientific Reports* 8, 17302.

Card S., Johnson L., Teasdale S. and Caradus, J. (2016). Deciphering endophyte behaviour: The link between endophyte biology and efficacious biological control agents. FEMS *Microbiological Ecology*. 92 (8): 1–19.

Chen J., Li Q. X. and Song B. (2020). Chemical nematicides-recent research progress and outlook. *Journal of agriculture, food and chemistry*, 2020, 68, 44, 12175-12188. https//doi.org/10.1021/acs.jafc.0c02871.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control (Review). *Annual Review of Phytopathology*. 2002, 40, 221−249.

CIDP. (2023). County Integrated Development Plan III (CIDP III, 2023-2027). Change agenda for socio-ecnomic development and wealth creation for Nyandarua County. Republic of Kenya, County Government of Nyandarua. 371.

Clay, K., Shearin, Z. R. C., Bourke, K. A., Bickford, W. A. and Kowalski, K. P. (2016). Diversity of fungal endophytes in non-native *Phragmites australis* in the Great Lakes. *Biological Invasions* 18, 2703e2716.

Coyne, D. L., Nicol, J. M. and Claudius-Cole, B. (2007). Practical plant nematology: A field and laboratory guide. SP-IPM secretariat. *International Institute of Tropical Agriculture* (IITA), Cotonou, Benin, 32-38.

Cumagun, C. J. and Moosavi, M. R. (2015). Significance of Bio-control agents of Phytonematodes. In: Bio-Control Agents of Phytonematodes, Askary, T.H. and P.R. Martinelli (Eds.), CABI International, United Kingdom, ISBN-13: 9781780643755, pp: 50-78.

Dababat, A. A., Selim, M. E., Saleh A. A. and Sikora, R. A. (2008). Influence of Fusarium wilt resistant tomato cultivars on root colonization of the mutualistic endophyte *Fusarium oxysporum* strain 162 and its biological control efficacy toward the root knot nematode *Meloidogyne incognita*. *Journal of Plant Diseases and Protection*, 115(6): 273-278.

Dahlin, P., Eder, R., Consoli, E., Krauss, J., and Kiewnick, S. (2019). Integrated control of *Meloidogyne incognita* in tomatoes using fluopyram and *lilacinum* strain 251. *Crop Protection* 124:104874. Doi: 10.1016/j.cropro.2019.104874.

Daykin, M. E. and Hussey, R. S. (1985). Staining and histopathological techniques in nematology. In K. R. Barker, C. C. Carter, and J. N. Sasser (eds). An Advanced Treatise on *Meloidogyne*. Vol. II: Methodology (Raleigh: North Carolina State University Graphics), pp. 39-48.

De Silva, N. I., Brook, S. and Lumyong, S. (2018). Use of endophytes as agents. DOI:10.1016/j.fbr.2018.10.001. *Elsevier*. *Fungal biology reviews* 33(2019), 133-148.

Díaz-González S, Marín P, Sánchez R, Arribas C, Kruse J, GonzálezMelendi P, Brunner F, Sacristán S. (2020). Mutualistic fungal endophyte *Colletotrichum tofieldiae* Ct0861 colonizes and increases growth and yield of maize and tomato plants. *Agronomy*. 10(10):1493. Doi: 10.3390/agronomy10101493.

Ding, H., Yang, X. and Xu, L. (202). Analysis and comparison of tri-biological performance of fatty acid-based lubricant additives with sulfur. *Journal of Bioresource products* 5(2):134-142. https://doi.org/10.1016/j.jobab.2020.04.007.

Dong, C., Wang, L., Li, Q. and Shang, Q. (2021). Epiphytic and endophytic fungal communities of tomato plants. *Horticultural plant journal*. 7(1):38-41.

Dorcas, N., Kalele, A., Affokpon, J., Coosemans, J. and Kimenju, W. (2010), "Suppression of root knot nematodes in tomato and cucumber using biological control agents". *African Journal of Horticutural Sciences*, 3: 17.

Ebone L. A., Kovaleski M. and Deuner C. C. (2019). Nematicides: History, mode, mechanism action. *Plant Science Today* 2019;6(2):91-97https://doi.org/10.14719/pst.2019.6.2.468.

Edel V., Steinberg C., Gautheron N., Recorbet G. and. Alabouvette C. (2001). Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *Microbiology Ecology*, 36: 61–71.

EL-Ashry, R. M., Ali, M. A. S., Elsoboki, A. E. A. and Aioub, A. A. A. (2021). Integrated management of M. incognita on tomato using combinations of abamectin, *Purpureocillium lilacinum*, rhizobacteria and botanicals compared with nematicide. *Egyptian journal of biological pest control*. (2021), 31.93. https://doi.org/10.1186/s41938-021-00438-x

Escobar C, Barcala M, Cabrera J, Fenoll C. (2015). Overview of root-knot nematodes and giant cells. *Advances in Botanical Research* 73, 1–32.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Feyisa B, Lencho A, Selvaraj T, Getaneh G. (2016). Evaluation of some botanicals and *Trichoderma harzianum* against root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato. *Journal of Entomology and Nematolology*. Vol.8 (2):11-18. https://doi.org/10.5897/JEN2015.0145

Forghan, F. and Hajihassani, A. (2020). Recent advances in the development of environmentally benign treatments to control Root-knot nematodes. *Frontiers of plant scince*.11.1125.doi:10.3389/fpls.2020.01125.

Ganeshan, K, Vetrivelkalai, P., Bhagawati, B., Gupta, N., Devrajan, K., Raveendran, M. and Balachandar D. (2021). Endophytic fungi as potential biocontrol agents agains troot-knot nematode *Meloidogyne incognita* in Banana. *Current journal of applied science and technology*. 40(29):7-18, 2021.

Gebreyohannes, L., Egigu, M. C., Manikandani, M. and Sasikumar, J. M. (2023). Allelopathic potential of *Lantana camara* L. leaf extracts and soil invaded by it on the growth performance of *Lipidium sativam* L. *Hindawi. The scientific world journal*, vol. 2023. Hhtps//doi.org/10.1155/2023

Gioia L., D'Errico g., Sinno M., Ranesi M., Woo S. L. and Vinale F. (2020). Survey of endophytic fungi associated with high-risk plants imported for ornamental purposes. Review. *Agriculture* 2020, 10,643; doi: 10.3390/agriculture 10120643.

Grabka, R., d'Entremont, T.W., Adams, S.J., Walker, A.K., Tanney, J.B., Abbasi, P.A. and Ali, S. (2022). Fungal Endophytes and Their Role in Agricultural Plant Protection against Pests and Pathogens. *Plants*, 2022, 11, 384, https://doi.org/10.3390/ plants11030384 *gy*. 8:11–18.

Guevara-Suarez, M.; Cárdenas, M.; Jiménez, P.; Afanador-Kafuri, L.; Restrepo, S. (2022). *Colletotrichum* Species Complexes Associated with Crops in Northern South America: A Review. *Agronomy*, 2022, 12, 548. https://doi.org/ 10.3390/agronomy12030548.

Herrera-Parra, E., Cristóbal-Alejo, J., and Ramos-Zapata, J. A. (2017). Trichoderma strains as growth promoters in *Capsicum annuum* and as biocontrol agents in *Meloidogyne incognita*. *Chilean Journal of Agricultural Research*. 77, 318–324. doi: 10.4067/S0718-58392017000400318.

Herrera, S. D., Grossi, C., Zawoznik, M. and Groppa, M. D. (2016). Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiological research*. 186, 37-43.

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell R. J. (2016). Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell*. 165(2):464–474. doi:10.1016/j.cell.2016.02.028.

Holbrook, C. C., Knauft, D. A. and Dickson, D. W. (1983). A technique for screening peanut resistance to *Melodogyne arenaria*. *Plant Disease*, 67:957-958

Hooper, D. J., Hallmann, J. and Subbotin, S. A. (2005). Methods for extraction, processing and detection of plant and soil nematodes. Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edition, 53-86.

Hussey, R.S., Barker, R.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Report*. 57, 1025–1028. https://ci. nii.ac.jp/naid/10012535898/.

Jaetzold, R., Schmidt, H., Hornetz, B. and Shisanya, C. (2010). Farm Management Handbook of Kenya, Vol. II, Natural Conditions and Farm Management Information. 2nd Edition, Part II/B Central Kenya, Subparts B1 and B2 Central and Rift Valley Province Middle and Ministry of Agriculture, Kenya, in Cooperation with the German Agency for Technical Cooperation (GTZ), Nairobi.

Jones, J. T., Haegeman A., Danchin, E. G. J., Gaur, H. S., Helder, J., Jones, M. G. K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., Wesemael, W. I. M. M. L. and Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14:946–961.

Jones R. K., Fourie H., Spaull V. W., Daneel M. S. and Waele D. (2017). Nematology in South Africa: A View from the 21st Century. © *Springer* International Publishing Switzerland. 2017. https://doi.org/ 10.1007/978-3-319-44210-5.

Kariuki, A. N., Waceke, J. W. and Mwangi, M. (2021). Efficacy of selected local fungal isolates in the management of root-knot nematode (*Meloidogyne* spp.) on tomato (*Solanum lycopersicum*) in Kenya. *Plant pathology journal*, *20*(*1*): 41-53, 2021.

Karuri, H. W., Olago, D., Neilson, R., Mararo, E. and Villinger, J. (2017). A survey of root knot nematodes and resistance to *Meloidogyne incognita* in sweet potato varieties from Kenyan fields. *Crop Protection*, *92*, 114–121.

Kerry B. (1997). Biological control of nematodes: Prospects and opportunities. Plant nematode problems and their control in the near east region, 79-92.

Khan, R. A. A., Najeeb, S., Mao, Z., Ling, J., Yang, Y., Li, Y., Xie, B. (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematodes. *Micro-organisms* 8, 401. https//doi.org/0.3390/micro-organisms 8030401.

Kibunja J. W. (2015). Effect of selected endophytic fungi and resistant tomato cultivars on *meloidogyn*e spp. in the coastal region, Kenya. PhD Thesis, Kenyatta University, Nairobi, Kenya.

Kimenju, J. W., Mweke, A. N, Mutitu, E. W. and Mutua, G. K. (2010). Poor hosts of root-knot nematodes and their applications as rotation crops in Okra production. *African journal horticultural sciences* (2010)3: 63-71

Kiriga, A. W., Haukeland, S., Kariuki, G. M., Coyne, D. L. and Beek, N. V. (2018). Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. *Biological control*, 119, 27-32. Doi:10.1016/j.biocontrol.2018.01.005.

Köhl J., Postma J., Nicot P., Ruocco M. and Blum B. 2011. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. *Biological Control*. 57(1):1-2.

Kubheka, B. P., & Ziena, L. W. (2022). *Trichoderma*: A biofertilizer and a bio-fungicide for sustainable crop production. DOI: 10.5772/intechopen.102405.

Kumar, A. P., Murali, V., Ngaraju, K. and Srinivas, M. (2023). Fungal endophytes: A potential application in integrated plant health management. *International journal of plant and soil science*. Vol. 35(18): 1570-1578. https:// doi.org/ 10.9734/ ijpss/2023/ v35:183427.

Kumar, K.K.; Dara, S. K. (2021). Fungal and bacterial endophytes as microbial control agents for plant-parasitic nematodes. *International Journal of Environmental Research and Public Health*, 2021, 18, 4269.

Lamovšek, J., Urek, G., and Trodan. S. (2013). Biological control of root-knot nematodes (*Meloidogyne* spp.): Microbes against the pests. *Acta Agriculturae Slovenica*, 101(2): 263–275.

Larran, S., Simon, M. R., Moreno, M. V., Siurana, M. S. and Perello, A. (2016). Endophytes from wheat as biocontrol agents against tan spot disease. *Biological Control,* 92, 17e23.

Latz, A. C. M., Jensen, B., Collinge, B. D. and Jorgensen, H. J. L. (2018). Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology and Diversity*. DOI:10:1080/17550874.2018.1534146.

Le, H. T. T., Padgham, J. L. and Sikora, R. A. (2009). Biological control of the rice root-knot nematode *Meloidogyne graminicola* on rice, using endophytic and rhizosphere fungi. *International Journal of Pest Management*, 55 (1): 31–36. DOI; 1080/09670870802450235.

Lecomte, C., Alabouvette, C., Edel-Hermann, V., Robert, F. and Steinberg, C. (2016). Biological control of ornamental plant diseases caused by *Fusarium oxysporum*: A review. *Biological control*, 101, 17e30.

Liu H., Zou W. X., Meng J. O., Hu J. and Tan R. X. (2000). New bioactive metabolites produced by *Colletotrichum* sp. an endophytic fungus in *Artemisia annua*. Plant science, 2000, 151, 67-73 (CrossRef).

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann. and S., Kahmann, R. (2015). Fungal effectors and plant susceptibility. Annual Review. *Plant Biology*. 66, 513e545.

Lugtenberg, B. J. J, Caradus J. R. and Johnson L. J. (2016). Fungal endophytes for sustainable crop production. *FEMS Microbiological Ecology*. 92 (12).fiw194.

Luna, J. (1998). Nematode suppression by cover crops. Crop rotation and cover crops suppress nematodes in potatoes. Department of Horticulture, Oregon State University.

Mahloatjie M., Vuyisile S. T., and Fhatuwan N. M. (2019). Nematocidal activity of fermented extracts from *Lantana camara* plant parts against *Meloidogyne javanica* on tomato. *International journal of vegetable science*. 27:1, 20-28. Doi:10.1080/193i5260.2019.1697981.

Maleta, C. M. (2011).Biology and ecology of the root-knot nematodes *Meloidogyne hispanica*: A species of emerging importance. PhD Thesis, university of Coimbra, Portugal.

Marra LM, Crfs S, Oliveira SM, Ferreira PAA, Soares BL, Carvalho RF, Lima JM, Moreira FMS. 2012. Biological nitrogen fixation and phosphate solubilization

by bactéria isolated from tropicals soils. *Plant and soil*. 357:289, doi: 10.1007/ s11104- 012-1157-z.

Meyer, S. L. F., Patchett, B. J., Gillanders, T. J., Kantori, M. R., Timper, P., Macdonald, M. H. (2020).Festulolium and fungal endophyte associations: Host status for *Meloidogyne incognita* and nematotoxic plant extracts. *Journal of nematology*. 2020; 52:le2020-76.

Mitkowski, N.A. and G.S. Abawi. (2003). Root-knot nematodes. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2003-0917-01. *Revised 2011*.

Muriithi, C., Matiri, F., Kihanda, F., Maina, D. & Kasungo, F. (2013). Evaluation of potential of tree tomato production and marketing in Eastern Kenya for improved nutrition and food security. *Joint Proceedings of the 27th Soil Science Society of East Africa and the 6th African Soil Science Society Conference*.

Musango, J. K. (2021). Potential of deploying indigenous fungi in the management of potato cyst nematode (*Globodera* spp.) on potato in Nyandarua County, Kenya. PhD Thesis, Kenyatta University, Nairobi, Kenya.

Naing, L., Winn, T., & Rusli, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, 1, 9–14.

Nassimi, Z. and Taheri, P. (2017). Endophytic fungus *Piriformospora indica* induced systemic resistance against rice sheath blight via affecting hydrogen peroxide and antioxidants. *Biocontrol Science Technology*. 3157: 1–16.

Navia D, Delgado A, Viera W, Báez F. and Trevor, J. (2017). Application of Bio-Products in Ecuadorian Agriculture: Case Banana. *International Journal of Clinical and Biological Sciences*, 2 (2): S7.

Naz, I., Khan, R. A. A., Masood, T., Baig, A., Siddique, I. and Haq S. (2021). Biological control of root-knot nematode, *Meloidogyne incognita*, *in vitro*, greenhouse and field in cucumber. *Biological control*, 152(2021)104429. https.//doi.org/10.1016IJ.biocontrol. 2020.104429.

Nchore, S.B., Waceke, J. W., Buttner, C., Omwoyo, O., and Ulrichs, C. (2016). Efficacy of soil solarization and selected organic amendments for the control pf root-knot nematodes in African nightshades. *RUFORUM Technical report* (ISSN 1607-9345) No. 14(1):771-777.

Niranja, S. R., Lalitha, S. and Hariprasad, P. (2009). Mass multiplication and formulation of biocontrol agents for use against *Fusarium* wilt of pigeon pea through seed treatment. *International journal of pest management*. 55(4):317-324.

Noling, J. W. (2000). Nematode management in tomatoes, peppers and eggplant

Oliveira J.A.S., Polli A.D., Polonio J.C., Orlandelli R.C., Conte H, Azevedo J. L., Pamphile J. A.(2020). Bioprospection and molecular phylogeny of culturable endophytic fungi associated with yellow passion fruit. Acta Sciences, *Biological Sciences*. 42(1):e48321. doi:10.4025/actascibiolsci.v42i1.48321.

Onkendi, E. M., Kariuki, G. M., Marais, M. and Moleleki, L. N. (2014). The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: A review. *Plant Pathology*, Pp 1- 11.

Oono, R., Lefevre, E., Simha, A. and Lutzoni, F. (2015). A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*). *Fungal Biology*. 119 (10), 917e928.

Pedrosa A. G. S. (2016). Genetic variability analysis of tamarillo (*Solanum betaceum* Cav.) and optimization of micropropagation conditions. University of Coimbra.

Pili, N.N. (2016). Possibilities of endophytic fungi from Kenya for growth promotion of rice (*Oryza sativa* L.) and defense against nematodes. PhD thesis, Ghent University, Ghent, Belgium.

Potshangbam, M., Devi, S. I., Sahoo, D. and Strobel, G. A. (2017). Functional Characterization of Endophytic Fungal Community Associated with *Oryza sativa* L. and *Zea mays* L. *Frontiers of Microbiology*. 8, 325e330.

Poveda J., Abril-Urias P., and Escobar C. (2020) Biological Control of Plant-Parasitic Nematodes by Filamentous Fungi Inducers of Resistance: *Trichoderma*, Mycorrhizal and Endophytic Fungi. https://doi.org/10.3389/fmicb.2020.00992.

Prohens, J. & Nuez, F. (2001). The Tamarillo (*Cyphomandra betacea*) A Review of a Promising Small Fruit Crop. *Small Fruits Review*, *1*(2), 43–68.

Qin, Z., Zhang, J. E., Ditommasa A., Wang, R. I. and Liang K. M. (2016). Predicting the potential distribution of *Lantana camara* L., under R.C.P scenario using ISI-MIP models. *Climate change*. 134:193-208.

Quesenberry, K. H., Baltensperger, D. D., Dunn, R. A., Wilcox, C. J. and Hardy, S. R. (1989). Selection for tolerance to root-knot nematodes in red clover. *Crop science*, 29:62-6.

Rana, M. K. and Brar, N. S. (2019). Tree tomato. *Vegetable crop science*. DOI: 10.1201/9781315116204-101. 915-922.

Ramírez-Gil, J. G., Gil-Aguirre, A. & Morales-Osorio, J. G. (2017). Etiología de enfermedades del cultivo de tomate de árbol (*Solanum betaceum* CAV.). *Revista de Protección Vegetal*, *32*(1), 33–51.

Saitou N. and Nei M. (1987). The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Schouten, A. (2016). Mechanism involved in nematode control by endophytic fungi. *Annual review of phytopathology*, 2016. 54:121-142.

Schulz, B., Haas, S., Junker, C., Andree, N. and Schobert, M. (2015). Fungal endophytes are involved in multiple balanced antagonisms. *Current Science*. 109, 39-45.

Sharon, E., Chet, I., Viterbo, A., Bary-Eyal, M., Nagan, H., Samuels, G. J. and Spiegel, Y. (2007). Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. *European Journal of Plant Pathology*, 118: 247–258.

Shaukat, S. S., Siddiqui I.A., Khan, G. H., Zaki, M. J. (2002). Nematicidal and allelopathic potential of *Argemone mexicana*, a tropical annual weed. *Plant and Soil*, 245: 239-447.

Shawky, S. M., Khalil, A. E., and Manal, M. S. (2010). Non-chemical control of root-knot nematode; *Meloidogyne javanica* on peanut in Egypt. *Zagazig Journal of Agricultural Research*, 37(1): 1-22.

Sikora, R. A. and Fernandez, E. (2005). Nematode parasites of vegetables. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, *2*, 319–392.

Silva Santos, S. D., Silva, A. A. D., Polonio, J. C., Polli, A. D., Orlandelli, R. C., Oliveira, J. A. D. S., Filho J. U. T. B., Azavedo J. L. and Pamphile, J. A. (2022).

Influence of plant growth-promoting endophytes, *Colletotrichum siamense* and *Diaporthe masirevici* on tomato plants (*Lycopersicon esculentum* Mill.). *Mycology*, *13*(4):257-270.https://doi.org/10.1080/21501203.2022.2050825.

Singh, C., Sharma, N. and Singh, B. R. (2016). *Trichoderma harzianum*: Mass multiplication and its interactions with different fungicides. *Journal of Biotechnology and Biosafety*. 4: 332-338.

Singh, S. K. & Khurma, U. R. (2007). Susceptibility of six tomato cultivars to the root-knot nematode, *Meloidogyne incognita*. *The South Pacific Journal of Natural and Applied Sciences*, *25*(1), 73–77.

Skider, M. M. and Vestergard, M. (2020). Imacts of root metabolites on soil nematodes. *Frontiers in plant science*, 10:1792. https://doi.org/10:3388/fpls.2019.01792

Solis, M. J. L., Cruz, T. E. D., Schnittler, M. and Unterseher, M. (2016). The diverse community of leaf-inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina*, *F. elastica* and *F. religiosa*. *Mycoscience* 57, 96-106.

Spadaro D. and Gullino M, L. 2005. Improving the efficacy of biocontrol agents against soil borne pathogens. *Crop Protection*. 24 (7): 601–613.

Stirling, G. R. (2018). "Biological control of plant-parasitic nematodes," in *Diseases of Nematodes*, eds G. O. Poinar and H. -B. Jansson (Boca Raton, FL: CRC Press), 103–150. doi: 10.1201/9781351071468.

Suebrasri T., Harada H., Jogloy S., Ekprasert J. and Boonlue S. (2020). Auxin-producing fungal endophytes promote growth of Sunchoke. *Rhizosphere* 16:100271.https://doi.org/10.1016/J. rhizosphere.2020.100271.

Sujatha, R., Vethamoni, P. I., Manivannan, N. & Sivakumar, M. (2017). Screening of Tomato Genotypes for root-Knot nematode (*Meloidogyne incognita* Kofoid and White Chit wood). *International Journal of Current Microbiolology and Applied Sciences*, *6*(3), 1525–1533.

Suri H. (2011) Purposeful sampling in qualitative research synthesis. *Qualitative Research Journal*, 11:63–75. Doi: 10.3316/QRJ1102063.

Talukdar R, Padhi S, Rai AK, Masi M, Evidente A, Jha DK, Cimmino A, Tayung K (2021) Isolation and characterization of an endophytic fungus *colletotrichum coccodes* producing tyrosol from *houttuynia cordata* thunb using ITS2 RNA secondary structure and molecular docking study. *Frontiers of Bioengineering and Biotechnology*. https://doi.org/10.3389/ fbioe.2021.650247.

Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* 9:678-687.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version, 11: *Molecular Biology and Evolution.* https://doi.org/10.1093/molbev/msab120.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*. 2007; 24(8): 1596–9.

Taye W, Sakhuja P. K and Tefera T. (2012). Evaluation of plant extracts on infestation of root-knot nematode on tomato (*Lycopersicon esculentum* Mill). *Journal of Agricultural Research and Development*. 2(3):86-91.

Thakur, S., Kumar, M. and Sharma M. K. (2020). Integrated nematode management: A comprehensive approach against root-knot nematode, *Meloidogyne incognita* infecting cucumber under protected cultivation. *Journal of pharmacognosy and phytochemistry*. JPP 2020: 9(6):1200-1204.

Tourey, M., Cimon, H., Gulsen, S. H., Ulug, D., Erdogus, D., Shapiro-Ilan, D. and Haziri, S. (2021). The impact of chemical nematicides on entomopathogenic nematode survival and infectivity. *Journal of nematology*. e2021/volume 53. https://doi.org/10.21307/jofnem-2021-049.

Touray, M., Gulcu, B., Ulug, D., Gulsen, S. H., Cimen, H., Kaya, H. K., Cakmak, I. and Haziri, S. (2020). Evaluation of different sponge types on the survival and infectivity of stored entomopathogenic nematodes. *Journal of invertebrate pathology* 171:107332.

Tranier, M. S., Pognant- Gros, J., Quiroz, R. C., González, C. N. A., Mateille, T. and Roussos, S. (2014). Commercial biological control agents targeted against plant-parasitic root-knot nematodes. *Brazilian Archives of Biology and Technology*, 57(6): 831–841.

Wada S. and Toyota K. (2008). Effect of three organophosphorous nematicides on non-target nematodes and soil microbial communities. *Microbes and Environment*, 23(4):331-336.

Waswa, S. J. (2021). Occurrence of phytonematodes and Biocontrol of *Meloidogyne* spp. (Root-knot nematodes) on Tree Tomato (*Solanum betaceum* Cav.) in Kiambu and Embu Counties, Kenya. M.sc Thesis, Kenyatta University, Nairobi, Kenya.

Waswa S, J., Waceke J. W. and Nchore S. B. (2020). Diversity of Plant Parasitic Nematodes of Tree Tomato (*Solanum betaceum* Cav.) in Kiambu and Embu Counties, Kenya-*Middle East Journal of Agriculture Research. EISSN: 2706-7955 ISSN: 2077-4605 DOI: 10.36632/mejar/2020.9.3.47 Volume: 09 | Issue: 03| July-Sept. | 2020 Pages: 605-616.*

Watanabe T. (2010). Pictorial Atlas of Soil and Seed Fungi: Morphologies of cultured fungi and keys to species. Third edition. CRC press, Taylor and Francis group. Florida, USA.

White, T. J., Bruns, T., Lee, S. and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsk, J. J., White, T. J. (Eds). PCR protocols: a guide to methods and applications. Academic Press, Inc., New York, Pp 315–322.

Xiang, N., Lawrence, K. S., and Donald, P. A. (2018). Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: A review. *Journal of Phytopathology*. 166, 449–458. Doi: 10.1111/jph.12712.

Yao, Y., Huo, J., Ben, H., Gao, W., Hao, Y., Wang, W. and Xu, J. (2023). Biological efficacy of endophytic fungus, *Acremonium sclerotigenum*, against *M. incognita*

under *in vitro* and *in vivo* conditions. *Biologia*. https//doi.org/10.1007/s11756-023-01505-4.

Zhang, S., Gan, Y., Ji, W., Xu, B., Hou, B., and Liu, J. (2017). Mechanisms and characterization of *Trichoderma longibrachiatum* T6 in suppressing nematodes (*Heterodera avenae*) in wheat. *Frontiers of Plant Science*. 8:1491. doi: 10.3389/fpls.2017.01491.

Zhou S, Qiao L., Jayawardena R. S., Hyde K. D., Ma X., Wen T. and Kang J. (2019). Two new endophytic *Colletotrichum* spp. from *Nothapodytes pittosporoides* in China. MycoKeys 49: 1–14(2019). https://doi.org/10.3897/mycokeys.49.31904.

Zhao W. T., Liu Q. P., Chen H. Y., Zhao W., Gao Y. and Yang X. L. (2020). Two novel eremophylane acetophenone conjugates of *Colletotrichum gloeosporioides*, an endophytic fungus on *Salvia miltiorrhiza*: *Fitoterapia* 2020, 141,104474. (CrossRef).

Zhou W., Verma V. C., Wheeler T. A., Woodward J. E., Starr J. L. and Sword G. A. (2020). Tapping into the cotton fungal phytobiome for novel nematode biological control tools. *Phytobiomes Journal* 2020. 4:19-26. https://doi.org/10.1094PBIOMES-08-19-0043.Shortcommunication.

APPENDICES

Appendix 1: Turn-it-in report summary

PhD thesis 8th August version

ORIGINALITY REPORT

14%	10%	9%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

Submission date: 08-Aug-2024 11:55AM (UTC+0300)

Submission ID: 2417171179

File name: PhD_THESIS_FINAL_DRAFT_AUGUST_20248th_August.docx (7.96M)

Word count: 31522

Character count: 183561

Appendix 2: Research publications and conference presentation

The list below indicates the papers that have been published from this work and conference attended:

1. Waswa, Stanlous Juma, Waceke, J. Wanohi and Maina, M. (2024). ***In-vitro* Nematicidal Efficacy of *Lantana camara* Leaf extract combined with Endophytic fungus (*Colletotrichum nigrum*) against Root-knot Nematodes**. *International Journal of Agriculture and Environmental Research*. ISSN: 2455-6939. Volume: 10, Issue: 06 November-December 2024 Page 842-860. DOI:https://doi.org/10.51193/IJAER.2024.10604.
2. Waswa, Stanlous Juma, Waceke, J. Wanohi and Maina, Mwangi. (2024). **Compatibility of Velum® (Synthetic Nematicide) with Endophytic *Colletotrichum nigrum* and Commercial Trichoderma asperellum against Root-Knot Nematodes on Tree Tomato**. *International Journal of Plant & Soil Science*, Volume 36, Issue 11, Page 278-292, 2024; Article no.IJPSS.126494 ISSN: 2320-7035. DOI: https://doi.org/10.9734/ijpss/2024/v36i115143
3. Waswa, Stanlous Juma, Waceke, J. Wanohi and Maina, Mwangi. (2024. **Integrated Management of Root-knot Nematodes on Tree Tomato**. *Elsevier, Rhizosphere*. *Rhozosphere* 33 (2025) 101012. https://doi.org/10.1016/j.rhisph.2024.101012.
4. 16th-19th Sept. 2024: **African Crop Science Conference** at Eduardo Mondlane University Main Campus Maputo, Mozambique: Stanlous J. Waswa, J. W. Waceke and M. Mwangi: Compatibility of Endophytic Fungus (*Colletotrichum*

nigrum) With Velum ® (Synthetic Nematicide) Against Root-Knot Nematode (*Meloidogyne* spp.) On Tree Tomato.

Appendix 3: Research authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: A99/21198/21

Date: 21st September, 2022

The Director General,
National Commission for Science, Technology & Innovation,
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR WASWA S. JUMA - REG. NO. A99/21198/21

I write to introduce Juma who is a Postgraduate Student of this University. The student is registered for a Ph.D. degree programme in the **Department of Agricultural Sciences & Technology in the School of Agriculture & Enterprise Development.**

Juma intends to conduct research for Ph.D. thesis entitled, **"Antagonistic Potential of Local Endophytic Fungi against Root-Knot Nematodes (*Meloidogyne spp.)* on Tree Tomato (*Solonum betaceum*) in Nyandarua County, Kenya".**

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

RM/cao

Appendix 4: Approval of research proposal letter

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 21st September, 2022

TO: Waswa S. Juma
C/o Department of Agricultural Science & Tech.
KENYATTA UNIVERSITY

REF: A99/21198/21

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting **14th September, 2022** approved your Ph.D. Research Proposal entitled **"Antagonistic Potential of Local Endophytic Fungi against Root-Knot Nematodes (*Meloidogyne spp.)* on Tree Tomato (*Solonum betaceum*) in Nyandarua County, Kenya".**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking and Progress Report Forms. The Forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D. studies.

Thank you.

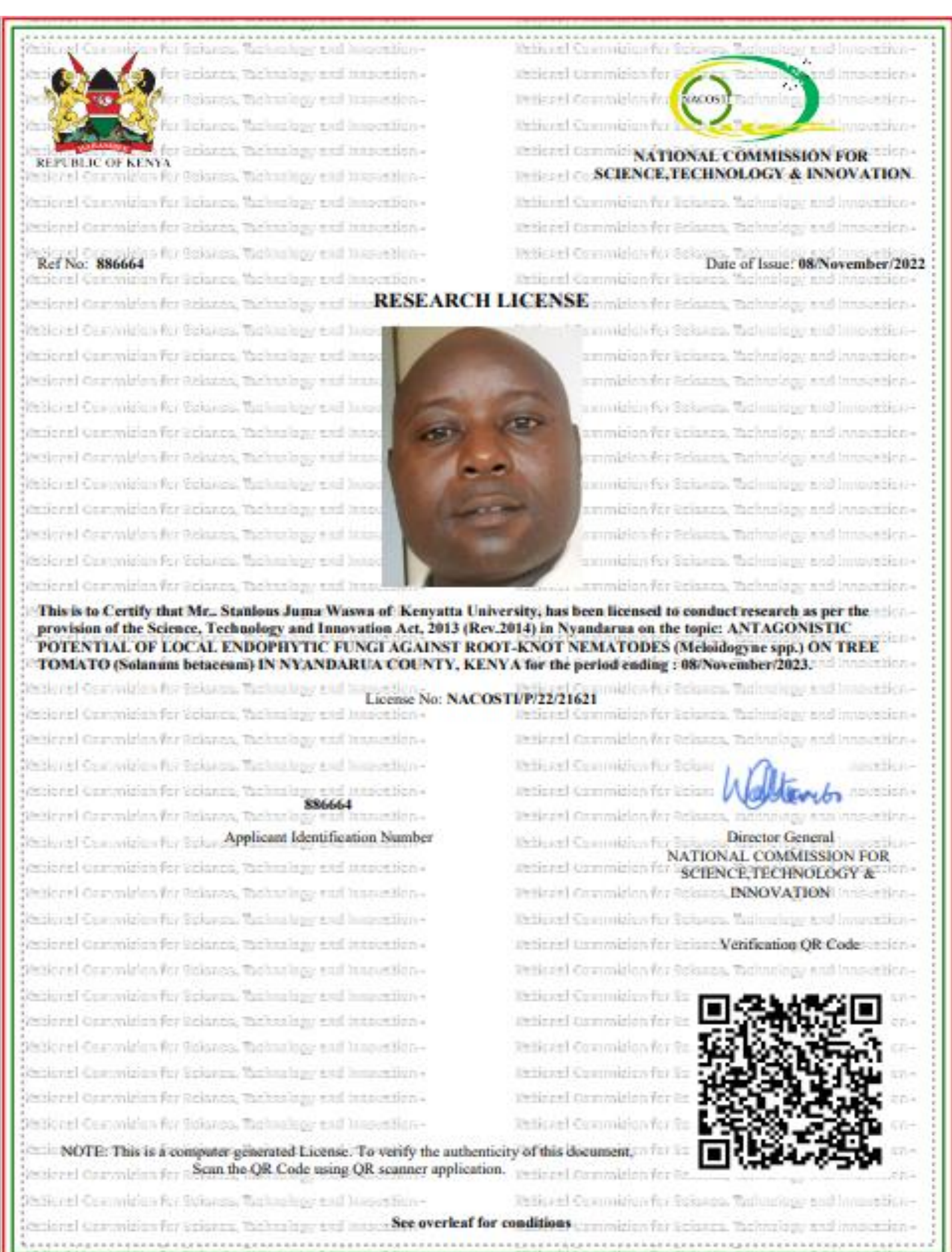
REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Agricultural Science & Technology
Registrar (Academic) Att; Mr. Richard Chweya
Supervisors:

1. Prof. Waceke Wanjohi
C/o Department of Agric. Sci. & Technology
KENYATTA UNIVERSITY

2. Prof. Maina Mwangi
C/o Department of Agric. Sci. & Technology
KENYATTA UNIVERSITY

Appendix 5: Research permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **886664**

Date of Issue: **08/November/2022**

RESEARCH LICENSE

This is to Certify that Mr.. Stanlous Juma Waswa of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nyandarua on the topic: ANTAGONISTIC POTENTIAL OF LOCAL ENDOPHYTIC FUNGI AGAINST ROOT-KNOT NEMATODES (Meloidogyne spp.) ON TREE TOMATO (Solanum betaceum) IN NYANDARUA COUNTY, KENYA for the period ending : 08/November/2023.

License No: **NACOSTI/P/22/21621**

886664

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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

See overleaf for conditions

Appendix 6: Morphological descriptions of endophytic fungal isolates

Twenty endophytic fungi that had more than 50 % mortality of J2s were identified using colony characteristics as described and shown below:

Colletotrichum nigrum (N2): The colonies on PDA appeared at first white with raised centre and changed to black with age on the front with irregular margins and sparse in density with no media alteration. The colony had pale cream white colour on the reverse. The margin colour was black (Plate N2 below).

Colletotrichum coccodes (N28): The colony appeared flat and grey black on both sides with irregular margin having dense black spores (Plate N28 below).

Colletotrichum coccodes (N41): The colony was sparse, appeared black on both sides of the PDA plate with raised centre and irregular margin (Plate N41 below).

Fusarium oxysporum f. sp *ciceris* (N5): The colony was pink-white to purple on the front with irregular margin and fast growing with cottony appearance on PDA media. The colour on the reverse appeared dark pink. The microconidia were oval while macroconidia were sparse and 3-4 septate (Plate N5 below).

Fusarium oxysporum (N11): The colony was pink-white to purple with irregular margin and fast growing with cottony appearance on PDA media. The microconidia were oval and numerous while macroconidia were curved slightly, sparse, and 3-4 septate (Plate N11 below).

Fusarium solani (N26): The colony appeared dense, cottony-white on the front side and cream-white on the reverse with irregular margin (Plate N26 below).

Fusarium fujikuroi (N36): The colony was flat, sparse and appeared white on both sides of the PDA plate with regular margin (Plate N36 below).

Aspergillus oerlinghausenensis (N10): Showed scattered colonies with raised margins having dark centre and whitish edges producing dark spores that quickly covered the PDA plate.The colonies were dark blue-green on the front and ginger –brown on the reverse (Plate N10 below).

Aspergillus spp. (N17): The colony was creamy-white on the front but yellow on the reverse side with irregular white margin. The colony was dense with media alteration from colourless to yellow (Plate N17 below).

Aspergillus ustus (N18): The colony appeared scattered with cream-yellow colour on the reverse while it appeared white on the front side (Plate N18 below).

Aspergillus spp. (N34): The colony appeared yellow on the reverse and cream white on the front side on the PDA plate. The surface appearance was cottony-white but with time changed colour to grey. The colony was dense with irregular margin and changed media from colourless to yellow with concentric rings (Plate N34 below).

Aspergillus fumigatus (N43): The colonies appeared yellowish-brown on the reverse and grey on the front side. The colonies were dense with concentric rings and irregular margins and had grooves radiating from the centre (Plate N43 below).

Microsphaeropsis arudinis (N14): The colony was cream white on the reverse but white on the front side with raised centre. The colony was dense with regular margin (Plate N14 below).

Penicillium onobense (N20): Colony appeared green-grey on the front side and white on the reverse with grooves. The colony was densely powdery with white margin that was regular (Plate N20 below).

Penicillium janthinellum (N24): Colony appeared as grey-yellowish with small grooves. The colony appeared scattered on the PDA plate appearing faint yellow on the reverse side but white on the front side (Plate N24 below).

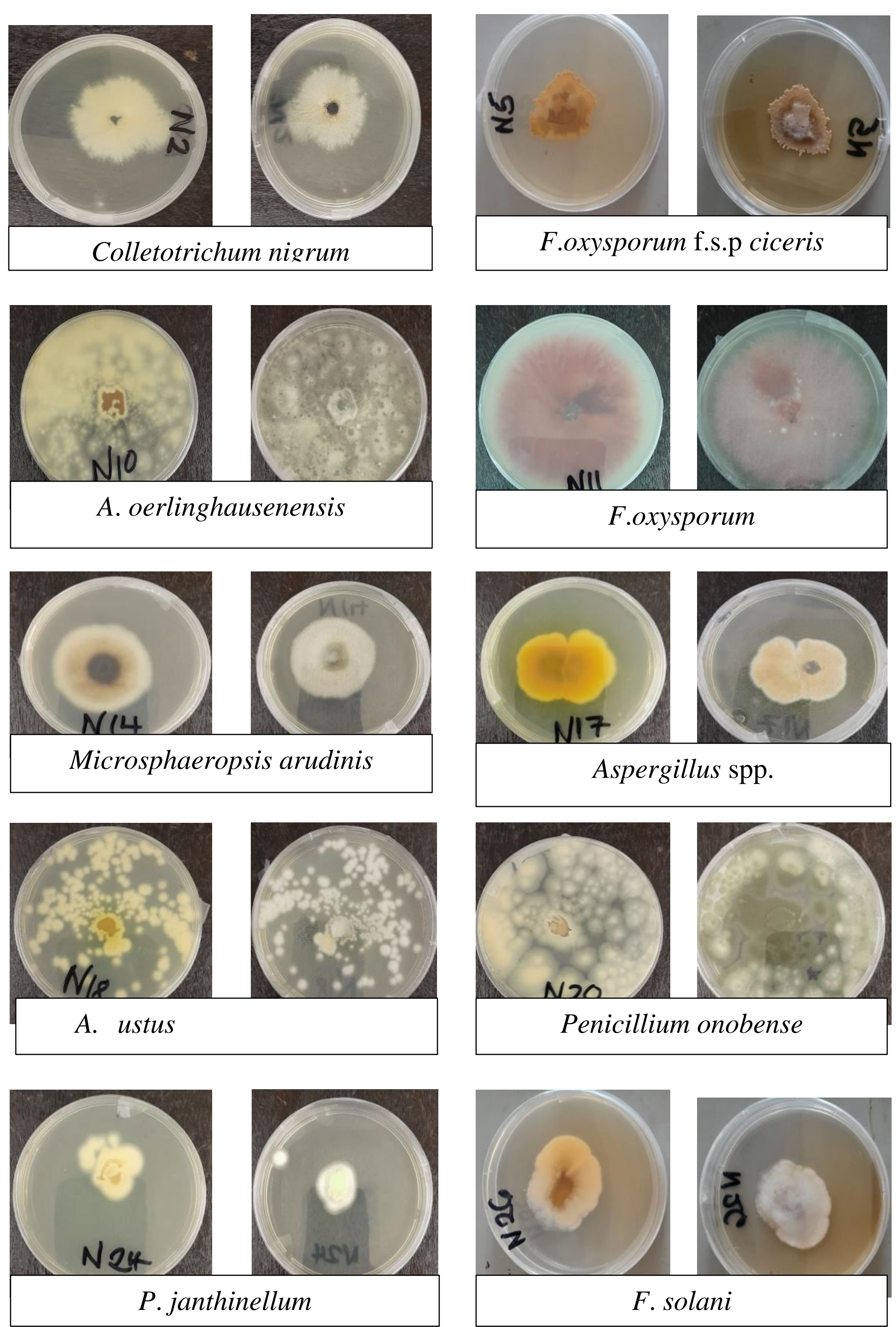
Rhizoctonia solani (N27): The colony was flat, appeared brown-yellow on the reverse and brown on the front side on PDA media with moderate density having irregular margin. The colony with time altered PDA media from coloulrless to yellow. The colony margin was yellow (Plate N27 below).

Didymella bellidis (N29): The colony appeared dense and white pink on the front while it was pink on the reverse side on PDA plate with irregular margin (Plate N29 below).

Didymella bellidis (N33): The colony appeared dark purple on the reverse and grey on the front side on the PDA plate with irregular margin. The colony was dense with a raised centre containing dark grey spores (Plate N33 below).

Periconia sp. (N31): The colony appeared white brown on the reverse and white on the front with a raised surface texture. The colony was dense and slow growing with irregular margin (Plate N31 below).

Purpureocillium lilacinum (N44): At first the colonies appeared white but changed to pink with time. The colony appeared pale brown on the reverse but pink on the front and scattered into smaller colonies with irregular margins but with raised centre (Plate N44 below).



Colletotrichum nigrum

F.oxysporum f.s.p *ciceris*

A. oerlinghausenensis

F.oxysporum

Microsphaeropsis arudinis

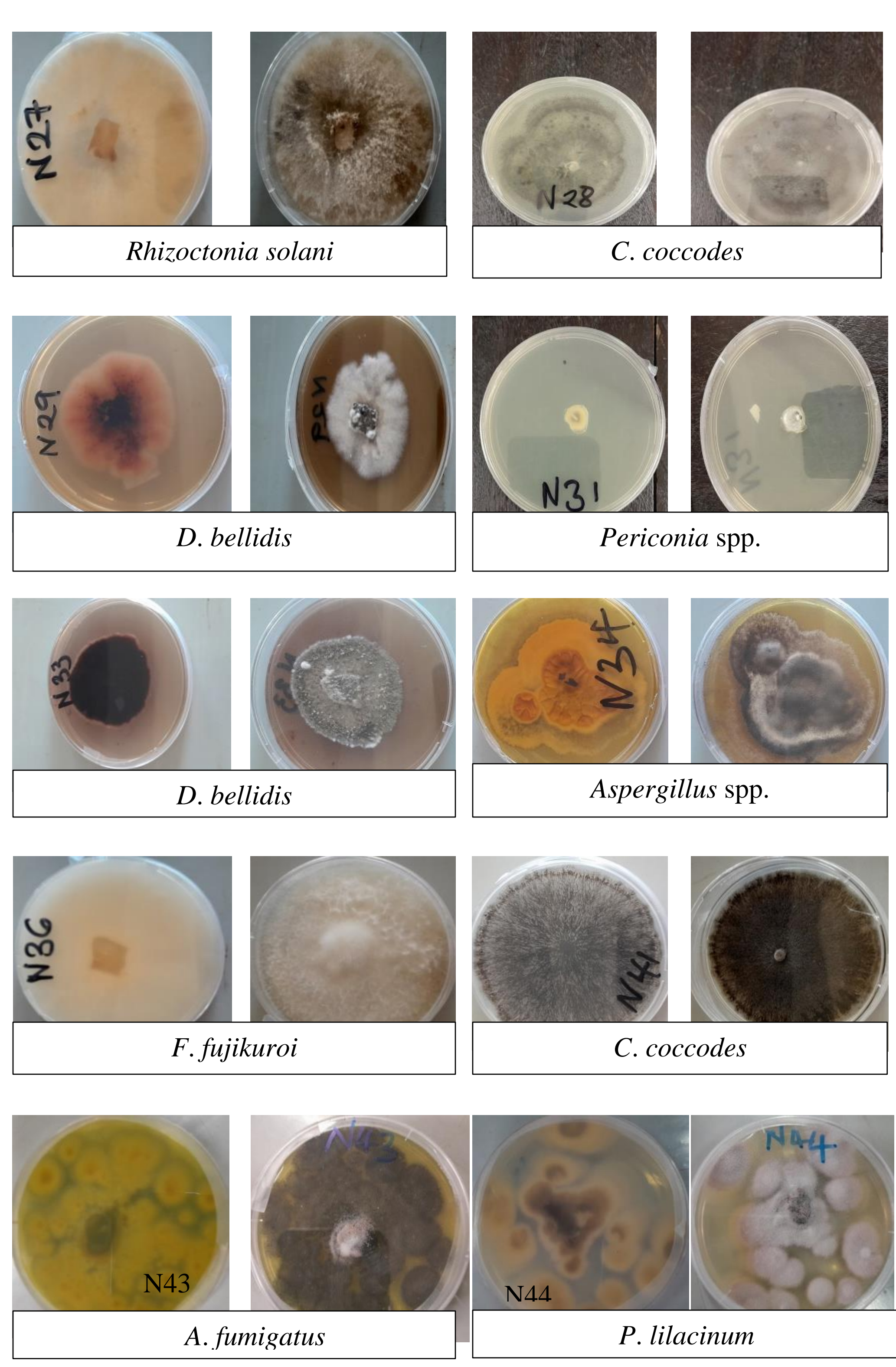
Aspergillus spp.

A. ustus

Penicillium onobense

P. janthinellum

F. solani



Colony characteristics (reverse and front) of endophytic fungal isolates

Appendix 7: ITS sequences for 18 endophytic fungi of tree tomato

>N2

TTCCGTAGGTGAACCTGCGGAGGGATCATTACTGAGTTACCGCTCTATAACC
CTTTGTGAACATACCTAACTGTTGCTTCGGCGGGCAGGGGGTGCCGCCTGCG
GACCCCCCTCCCGGCCCTGCCCTCACGGGCGGAGCGCCCGCCGGAGGATAC
CTAAACTCTATTTTAACGACGTTTCTTCTGAGTGGCACAAGCAAATAAATTA
AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGT
CATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGCGTTGATTCGT
AGGCCCTTAAAGGTAGTGGCGGACCCTCTCGGAGCCTCCTTTGCGTAGTAAC
TAACGTCTCGTCACTGGTATTCGGAGGGACTCTTGCCGTAAAACCCCCAAAT
TTTAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA
TCAATAAGCGGAGGAA

>N5

CGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCC
CTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAAC
GGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGA
GTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGG
GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGA
CTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCA
TAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTA
AACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA
CTTAAGCATATCAATAAGCGGAGGAA

>N10

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAAGTGAGGGCCCTCTGG
GTCCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCC
GTTTCGACGGCCGCCGGGGAGGCCTCGCGCCCCCGGGCCCGCGCCCGCCGA
AGACCCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCA
TAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGT
CCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCC
CCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCC
TCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCCAGCC
GACACCCAACTTTATTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCGGAGGAA

>N11

CCGGGAGATATTTCCGTAGGGTGAACCTGCGGAGGGATCATTACCGAGTTT
ACAACTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCC
CGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTC
TATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCC
CGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGC
ACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCG
GTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCG
TCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGT
AGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAG

>N14

CCCCGGAGCCCCGGCCCCCCCGTTGAATTGGTAAAGATCCTTCCGTAGGGTG
AACCTGCGGAAGGATCATTACCAATTCAACGGTGTGGTCGCGGCCTCCGGG
GGCTCCCCCCGGGCGGTAGAGGTAACACTCTTACGCGCCACATGTCTGAAT
CCTTTTTTTACGAGCACCTTTCGTTCTCCTTCGGTGGGGCAACCTGCCGTTGG
AACTTATCAAAACCTTTTTTGCATCTAGCATTACCTGTTCAGATACAAACAA
TCGTTACAACTTTCAACAATGGATCTCTTGGCTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGA
GCGTCATCTACACCCTCAAGCTCTGCTTGGTGTTGGGCGTCTGTCCCGCCTC
TGCGCGTGGACTCGCCCCAAATTCATTGGCAGCGGTCTTTGCCTCCTCTCGC
GCAGCACATTGCGCTTCAGAGGGGTGTGGGCCGCGTCCACGAAGCAACATT
ACCGTCTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC
AATAAGCGGAGGA

>N18

CGTAGGTGAACCTGCGGAAGGATCTTTACCGAGTGCAGGTCTGCCCCCGGG
CAGGCCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGC
GCCCCCCCGGGGGTAGCCGCCGGAGACCACACCGAACCTCCTGTCTTTAGT
GTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTT
GGTTCCGGCATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCA
TTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTTCAAGCCCGGCTT
GTGTGTTGGGTCGTCGTCCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCG
GCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCGATTAGG
GCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTCTATTTTACCAGGTTGACC
TCGGATCAGGTAGGGATACCCGCTRAACTTAAGCATATCAATAAGGGGAGG
A

N20

CTTCCGTAGGTGAACCTGCGGAAGGATCATTTACTGAGTGAGGGCCCTCTG
GGTCCAACCTCCCACCCGTGTTTATTGTACCTTGTTGCTTCGGCGAGCCCGC
CTCACGGCCGCCGGGGGGCACTTGCCCCCGGGCCCGCGCCCGCCGAAGACA
CCATTGAACTCTGTCTGAAGATTGCAGTCTGAGTAGATTAGCTAAATCAGTT
AAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC
GAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTT
GAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGT
CATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTTCGCCCCCCGTTCTTC
GGGGGGCGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCG
TATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCCCGCCGGCGAC
ACCCAAATCAATCTATCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCT
GAACTTAAGCATATCAATAAGCGGAGGAA

>N24

CTTCCGTAGGGTGAACCTGCGGAAGGATCATTACCGAGTTGCCAGGGCCCT
CTGGGTCCAACCTCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCC
CGCCGTTCCGGCCGCCGGGGGGCATCCGCCCCCGGGCCCGCGCCCGCCGAA
GACACCATTGAACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGCTAAATC
AGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAG
TCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA
GCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGCCCCCCGGC
TACCGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGA
GCGTATGGGGCTTCGTCACCCGCTCTGTAGGCCCGGCCGGCGCCCGCCGGC
GACCCCCCTCAATCTTTCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCGGAGGAA

>N26

CGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTATACAACTCCCAAACC
CCTGTGAACTTACCTATAACGTTGCTTCGGCGGGAACCGACGGCCCCGTAAC
ACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTTGTTATTTATATGTTTCT
CTGAGTAAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGC
TCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTC
TGGCGGGCATGCCTGTCCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCT
GGCGTTGGGGATCGGCGGAGCCCTCTGGGCACACGCCGCCCCCTAAATCTA
TTGGCGGTCTCGCTGCAGCCCACTGCGTAGTAGCTAACACCTCGCAACTGGA
AAGCGGCGCGGCCATGCCGTAAAACACCCAACTTTATGATGTTGACCTCGG
ATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

>N27

CTGAATTTAATGTAGAGTTGATTGGAGCTGACTCCCTCCGGGTTAATATTTT
TGGGTGCGCACGCCCCCCCTTTTTTTTCATCCCACACACACCTGTGAACCTG
TGAGATAGTAGTTGGGGACTTTAATGGACCCACTTCTGTCTACTTAATTCAT
ATAAAATCAATTTAATTTAAAACGAATGTAATGGATGTAACGCATCTAATA
CTAAGTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACCTTGCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTAT
CATGAAATCTTCAAAGTAAATCTTTTGTTAATTCAATTGGTTTCTACTTTGGT
ATTGGAGGGCATTGCAGCTTCACACCTGCTCCTCTTTGTCCATTAGCTGGAT
CTCAGTGTTATGCTTGGTTCCACTCAGCGTGATAAGTTATCTATCGCTGAGG
ACACTGTAAAAGGTGGCCAAGGTAAATGCAGATGAACCGTTTCTAATAGTC
CATTGACTTGGACAATAAATTAATTTTTATGATCTGATCTC

>N28

CGTAGGTGAACCTGCGGAGGGATCATTACTGAGCTCCCGCTCTATAACCCTT
TGTGAACATACCTAACTGTTGCTTCGGCGGGCAGGGGGTGCCGCCTGCGGA
CCCCCCTCCCGGCCCTGCCCTCACGGGCGGAGCGCCCGCCGGAGGATACCA
AACTCTATTTTAACGACGTTTCTTCTGAGTGGCACAAGCAAATAATTAAAAC
TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTT
CAACCCTCAAGCTCTGCTTGGCGTTGGGGCCCTACGGTTGACGTAGGCCCTT
AAAGGTAGTGGCGGACCCTCTCGGAGCCTCCTTTCCGTAGTAACTAACGTCT
CGCACTGGGATTCGGAGGGACTCTTACCGTAAAACCCCCAAATTTTTAAAG
GTTGACCTCGGATCAAGTAGGAATACCCGCTGAACTTAAGCATATCAATAA
GCGGAG

>N29

GGACCCGCCGAGGAAACGAAGGTACTCAACAGACATGGGTAAGATAGCAG
CCAACGCCTACAACTCTAGGTAAATTCTTTCCGTAGGGTGAACCTGCGGAA
GGATCATTACCTAGAGTTGTAGGGCTTTGCCTGCTATCTCTTACCCATGTCTT
TTGAGTACCTTCGTTTCCTCGGCGGGTCCGCCCGCCGATTGGACAATACTTA
AACCCTTTGTAATTGAAATCAGCGTCTGAAAAACTTTAATAGTTACAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTA
CCTTCAAGCTTTGCTTGGTGTTGGGTGTTTGTCTCGCCTCTGCGCGCAGACTC
GCCTCAAAACAATTGGCAGCCGGCGTATTGATTTCGGAGCGCAGTACATCT
CGCGCTTTGCACTCATAACGACGACGATCCAAAAGTACATTTTTACACTCTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCG
GAG

>N31

AGCTTTTGCATTTCCCCCAACTTATACSCACTTTCGCCCTTTTTTAAAGCGCA
AGCACCCAAACTCCATTGTTCAAACTGGCATAATTAGTTGTATAGAAGTTTC
GGAAGCCCACCGGTTTGTGTGTTCCGGCGGCGGCGGGCCGCCGGGAGGTTG
TGGGTACATATAGGCAAAGGCCAAAAGCAGCGCGCAACCCCCGGGGCCGG
GGCCGGTATTTTGTAATCCCTCCGTAGGGTGAACCTGCGGAAGGATCATTAC
AAAATACCTGCGCCGGCCCCCCGGGGTTGCGCGCTTCTATTTCACCCTTTGC
CTATGTGTACCCTCATCGCTTCCTCGGCAGGCCCGCCTGCCGCCAGGAACCA
CACAAAACCCTTGCATCTATACACGAAAACTTCTGATAACAACCTAAATTAT
TACAACTTTTAACAATGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACATTGCGCCCCTCGGTATTCCGTGGGGCACGCCTGTTCGAGCGT
CATTTACACCCTCAAGCCCAGCTTGGTGTTGGGCGTCTGTCCCGCTCCCGTA
GCGCGGACTCGCCTCAAAGTCATTGGCGGCGGTCGTGCCGGCTCCTCGCGC
AGCACATTTGCGCTTCTCGGAGGCCCGGCGGATCAGCACCCAGCAAGCTAC
ACTTTATGACTTGACCTCGGATCAGGTGAGGATACCCGCTGAACTTAAGCAT
ATCAATAAGCGGAGGAAGATCATTACAAAATACCTGCGCCGGCCCCGGGGT
TGCGCCTTCTATTTCACCTTTGCCTATGTGTACCTCATCCTTCTCGGCGGGCC
GCCTGCGCCGGAACACACAAACCCTTGCATTCTCCCGAAAACTTCTGATAAC
AACTAAACTTATTACACTTTTTAACAATGGGATCTCTTGGGTTTCTGGCATC
ATGAAAAAACGCAC

>N33

CTTCCGTAGGTGAACCTGCGGAAGGATCATTACCCCTAGAGTTGGTAGGGC
TTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACCTTCGTTTCCTCGGCGG
GTCCGCCCGCCGATTGGACAATACTTAAACCCTTTGTAATTGAAATCAGCGT
CTGAAAAACTTTAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGG

GCATGCCTGTTCGAGCGTCATTTGTACCTTCAAGCTTTGCTTGGTGTTGGGT
GTTTGTCTCGCCTCTGCGCGCAGACTCGCCTCAAAACAATTGGCAGCCGGCG
TATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGACGACG
ATCCAAAAGTACATTTTTACACTCTTGACCTCGGATCAGGTAGGGATACCCG
CTGAACTTAAGCATATCAATAAGCGGAGGAA

>N36

CCCGTCCCGTTTTCCCGGAGCGGATTTTCCGCGAGGCAACAATTGTATTTTC
GCAGGGTTGGGAGTTGTAAACTCGGTAAGACCTCCGTAGGGTGAACCTGCG
GAGGGATCATTACCGAGTTTACAACTTCCAAACCCCTGTGAACATACCACTT
GTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGA
GGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAA
ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAAT
CTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAG
CGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGC
GTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAA
ACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGA
ATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT
AAGCGGAGGAA

>N41

CTCCGTAGGTGAACCTGCGGAGGGATCATTACTCAGTTACCGCTCTATAACC
CTTTGTGAACATACCTAACTGTTGCTTCGGCGGGCAGGGGGTGCCGCCTGCG
GACCCCCCTCCCGGCCCTGCCCTCACGGGCGGAGCGCCCGCCGGAGGATAC
CAAACTCTATTTTAACGACGTTTCTTCTGAGTGGCACAAGCAAATAATTAAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA

ACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCAT
TTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGTTGACGTAGGCCC
TTAAAGGTAGTGGCGGACCCTCTCGGAGCCTCCTTTGCGTAGTAACTAACGT
CTCGCACTGGGATTCGGAGGGACTCTTGCCGTAAAACCCCCAAATTTTTAAA
GGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA
AGCGGAGGAA

>N43

TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGT
CCAACCTCCCACCcGTGTtTATcGTtCCTTGTTGCTTCGGCGGGCCCGCCGTTT
CGACGGTCtCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGAC
CCCAACATGAACGCTGTTCTGAAAGTATGCAGTTTGAGTTGATTATCGTAAT
CAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAAC
GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
GTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCG
AGCGTCATTGCTGCCCTCAAGCACGGTTTGTGTGTTGGGCCCCCGTCCCCCT
CTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCG
AGCGTATGGGGTTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGAC
ACCCAACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCT
GAACTTAAGCATATCAATAAGCGGAGGA

>N44

TAGGTGAACCTGCGGAGGGATCATTCCCGAGTTATCCACCTCCCACCCCCCC
TGTGACCCTTACCTCAGTTGCCTCGGCGGGAACGCCCCGGCCGCCTGCCCCC
GCGCCGGCGCCGGACCCAGGCGCCCGCCGCAGGGACCCCAAACTCTCTTGC
ATTACGCCCAGCGGGCGGAATTTCTTCTAGGAGTTGCACAAGCAAAAACAA
ATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC

GAATCTTTGAACGCACATTGCGCCCGCCAGCATTGGGGCGGGCATGCCTGTT
CGAGCGTCATTTCAACCCTCGAGCCCCCCCGGGGGCCTCGGTGTTGGGGG
ACGGCACACCAGCCGCCCCCGAAATGCAGTGGCGACCCCGCCGCAGCCTCC
CCTGCGTAGTAGCACACACCTCGCACCGGAGCGCGGAGGCGGTCACGCCGT
AAAACGCCCAACTTTCTTAGAGTTGACCTCGGATCAGGCAGGAATACCCGC
TGAACTTAAGCATATCAATAAGCGGAGGA