EFFICACY OF INDIGENOUS ANTAGONISTIC FUNGI AGAINST ROOT-KNOT NEMATODES ON TOMATO IN KIRINYAGA COUNTY, KENYA

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DECLARATION

I Anne Kariuki, declare that the work presented in this thesis is my original work and has not been submitted for award of a degree in any other University or any other ward.

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Signature: _____Date……

Supervisors' Approval,

We confirm that the work presented in this thesis was carried out by the candidate under our supervision as University supervisors.

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DEDICATION

I dedicate this study to my parents, Mr. & Mrs. Francis Kariuki for laying down the foundation to my success and all the support offered. To my husband, Jonah Kung'u who has been a source of inspiration and continually provided moral, emotional, spiritual and financial support.

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ABBREVIATIONS AND ACRONYMS

µm	micrometer
ANOVA	Analysis of Variance
BCA	Bio-Control Agent
CRD	Completely Randomized Design
DAI	Days After Inoculation
DAT	Days After Transplanting
LSD	Least Significant Difference
Min	Minutes
NaOCl	Sodium hypochlorite
PPN	Plant Parasitic Nematodes
RCBD	Randomized Complete Block Design
RF	Reproductive Factor
RKN	Root Knot Nematode
SAS	Statistical Analysis Software

ABSTRACT

Root-knot nematodes the *Meloidogyne* species are a serious threat to tomato production in both small and large scale tomato farms in Kenya. Management of the nematode is primarily dependent on the application of chemical nematicides. Chemical nematicides though very effective are expensive and also environmentally unfriendly due to their residual toxicity and pollution of the environment. The main aim of the current study was to improve tomato production through management of root-knot nematodes (RKN) using bio-control agents in Kirinyaga County, Kenya. The efficacy of antagonistic fungi in controlling RKN on tomatoes was evaluated *in vitro* and *in vivo*. Tomato root samples were obtained from Mwea. Fungal isolates were isolated from healthy tomato roots and *Meloidogyne* egg masses by direct plating technique. Root-knot nematodes inoculum was extracted by root maceration method from heavily galled tomato roots. A greenhouse experiment was set up in Kenyatta University and arranged in a Completely Randomized Design (CRD) with 4 replicates. A field with high nematode infestation was identified through soil sampling and analysis for presence of nematodes. The field trials were laid in a Randomized Complete Block Design (RCBD) with 4 replicates. The parameters evaluated included plant height, plant dry weight (roots and shoots), RKN egg hatch rate, juvenile mortality, juveniles' population in the soil, galling and egg mass indices, reproductive factor and fruit yield of tomatoes. Data collected was subjected to Analysis of Variance (ANOVA) using SAS software version 9.2 and means separated using Fisher's Least Significant Difference (LSD) test at 5% level of significance. A total of 45 fungal isolates (from roots and RKN egg masses) were identified and placed in various genera including *Trichoderma* spp., *Paecilomyces* spp. and *Fusarium* spp. using morphological characteristics and molecular techniques. *Trichoderma* spp. was the most prevalent (38%). All the isolated fungi had significant ($P \leq 0.05$) effect on egg hatch rate and juvenile mortality *in vitro*. The RKN egg hatch rate inhibition by the fungal isolates was notably ($P \leq 0.05$) higher than in the untreated control. *Trichoderma harzianum* 2 recorded the lowest egg hatch rate (22.33%) while the highest was in the untreated control (69.33%). The fungal isolates also caused RKN juvenile mortality that was significantly higher than in the untreated control. The highest juvenile mortality (91%) was caused by *T. harzianum* 2 after 10 days of exposure which was statistically ($P \leq 0.05$) greater than the untreated control (2%). The five selected fungal isolates tested *in vivo* significantly enhanced the growth and yield of tomato as well as reduced the RKN population and reproduction in the soil as compared to the untreated control. Among the tested isolates, *Trichoderma harzianum* 1 and *T. harzianum* 2 were the most effective in promoting growth in terms of increased shoot height, root length, plant dry weights and yield of tomatoes. The results of this study show that diverse fungi associated with tomato roots and RKN egg masses have potential of controlling RKN *in vitro* and *in vivo* as well as enhance growth and yield of tomato.

CHAPTER 1: INTRODUCTION

1.1 Background to the study

Tomato, (*Solanum lycopersicum* L.) is regarded a very important vegetable crop in Kenya and in the world (Waiganjo *et al*., 2013). It is of economic value as the crop provides domestic and nutritional requirement, generates revenue, earning foreign currency and also creates job opportunities (Singh et al., 2017). Tomatoes are the most important vegetable and accounts 37.63 percent in terms of value of the exotic vegetables (HCDA 2018). The acreage under production grew from 27,053ha in 2017 to 28,263 ha in 2018 a 4.5percent increase while production increased from 507,275tons in 2017 to 574,458tons in 2018 a 13.2percent increase. There was a 14.5 percent increase in value from Ksh. 17.38billion in 2017 and to 19.90 billion in 2018 (HCDA 2018).

The production, however, faces the challenge of pests and diseases (Maerere *et al*., 2006). The major diseases affecting tomatoes are bacterial wilt, early blight, late blight and tomato yellow leaf curl virus. The major pests of economic importance include Leaf miner, (*Tuta absoluta*); white flies, (*Bemisia tabaci*); aphids, (*Aphis gossypii*); mites, (*Tetranychus urticae*), thrips, (*Frankliniella occidentalis*), bollworms, (*Helicoverpa armigera*) and root-knot nematodes (*Meloidogyne* spp.) (Varela *et al*., 2003; Waiganjo *et al*., 2008).

Root-knot nematodes (*Meloidogyne* spp.) are present in various regions of the world and affect a variety of crops which makes their control difficult. Plant parasitic nematodes can severely damage vegetables leading to yield losses worldwide (Karssen and Moens, 2006). Root-knot nematodes have taken the first position amid the five most

important plant pathogens. Similarly, they rank first among the key genera of plant parasitic nematodes in the world (Mukhtar *et al*., 2017; 2018).

The genus *Meloidogyne* is of concern to both small and large scale tomato growers (Kamran *et al*., 2011). They cause up to 5% global crops yield losses and in global perspective $157 billion are lost to root-knot nematodes (Cetintas and Yarba, 2010). Naika *et al*., 2005 reported that the *Meloidogyne* species can reduce tomato yield by 30% in the tropics. Besides the direct damage by root-knot nematodes to plants, they are also found in relationships with other pathogenic organisms like *Ralstonia solanacearum*. These associations result to disease complexes which in turn aggravate the wilt diseases (Aslam *et al*., 2017a, b).

The most effective techniques for control of *Meloidogyne* spp. have been the use of nematicides (Onkendi *et al.,* 2014). However, nematicides use is limited due to several shortcomings (Faruk *et al*., 2011). These drawbacks include high costs, resistance development in nematodes, health hazards, environmental pollution, residual toxicity and harmful effects on the soil microorganisms besides phytotoxicity. These nematicides have high toxicity to humans and animals and also cause environmental pollution (Nofal, 2009), seriously threatening ecosystems.

Tomato is the most favourable host for RKNs (Waiganjo *et al*., 2006); therefore, effective management of the pathogen is necessary for profitable tomato production (Kariuki *et al*., 2006). In Kenya, efforts to control RKN have employed the use of nematicides, bio-control agents, cultural practices and pest-resistant varieties. However, the management techniques have been used on a small-scale and irregular basis due to low level of awareness about nematodes (Kimenju *et al*., 2008).

Integrated nematode management including use of antagonistic micro-organisms has been considered as an alternative. In this aspect, studies to determine the nematicidal effect of different biological agents have been conducted. Some bacteria including; *Pseudomonas fluorescens* (Norabadi *et al*., 2014), *Bacillus firmus* and *Pasteuria penetrans* (Lamovšek *et al*., 2013) have also been identified. Studies on fungal antagonists have also been conducted including, *Arthrobotrys oligospora* (Kalele *et al*., 2010), *Paecilomyces lilacinus* and *Pochonia chlamydosporia* (Silva *et al*., 2017b) and *Trichoderma viride* and *Trichoderma harzianum* (Mukhtar, 2018).

1.2 Problem Statement

The tomato industry in Kenya is faced by a major challenge due to infestation by parasitic nematodes with *Meloidogyne* spp. alone causing 30-100% yield loss (Onkendi *et al*., 2014). The presence and damage of root knot nematode on smallholder tomato farms in Kenya, specifically in Mwea area, has been reported (Wabere, 2016). Continuous cultivation of susceptible cultivars has also increased nematode reproduction resulting in low yields (Corbett *et al*., 2011). Furthermore, RKN interacts synergistically with other pathogenic fungi and bacteria hence increase the damage that leads to great yield losses.

Although chemical nematicides have been effective in controlling *Meloidogyne* spp. (Onkendi *et al.,* 2014)., their high costs and harmful effects on people, animals and other beneficial microbes found in the soil limit their use (Faruk *et al*., 2011). Use of some broad spectrum nematicides including methyl bromide, has been restricted due to their toxicity to human beings and negative effects to the environment.

Most of the research done on the application of biological control methods has been conducted outside Africa (Forghani and Hajihassani, 2020). In Kenya, few studies have been conducted in regards to locally-isolated bio-control agents. Hence, there is a need to explore the local antagonistic fungi and test their efficacy against root-knot nematodes.

1.3 Justification of the study

Tomato is a valuable vegetable sold and consumed in Kenya. Kirinyaga, County is one of the major tomato growing regions and root-knot nematodes have been reported to infest tomatoes in these areas. The climatic conditions in the area favour reproduction and survival of root-knot nematodes. It is therefore important to consider sustainable management of root-knot nematodes in tomato farms. Although, chemical nematicides have proved effective against root-knot nematodes, concerns are raised due to their toxicity. These have prompted research for safer management alternatives such as biological control (Hussaini, 2014).

Biological control techniques are considered as viable alternatives to chemical control. Nematophagous fungi including *Aspergillus* sp., *Paecilomyces lilacinus* (Mukhtar *et al*., 2013), *Pochonia chlamydosporia* and *Trichoderma* sp. (Mukhtar *et al*., 2013; Al-Hazmi *et al*., 2015) have showed suppressive effects against nematodes including *Meloidogyne* genus. Effective fungal antagonists can be found in most agricultural soils and provide an inexpensive environmental friendly technique for management of parasitic nematodes. The effectiveness of antagonistic micro-organisms in management of plant nematodes has however been considered to be partial (Viaene *et*

al., 2006), hence should be applied in combination with other practices in order to maintain nematodes populations below economic thresholds.

This study therefore was necessary to assess the use of local bio-control agents in managing root-knot nematodes on tomato production in Kenya.

1.4 Objectives

1.4.1 General objective

To improve tomato production through management of root-knot nematodes using local bio-control agents in Kirinyaga County, Kenya.

1.4.2 Specific objectives

1. To isolate and identify fungal organisms associated with tomato and root-knot nematodes.
2. To evaluate the efficacy of the fungal isolates in managing root-knot nematodes *in vitro*.
3. To evaluate the efficacy of the selected fungal isolates in managing root-knot nematodes on tomatoes under greenhouse and field conditions.

1.5 Hypotheses

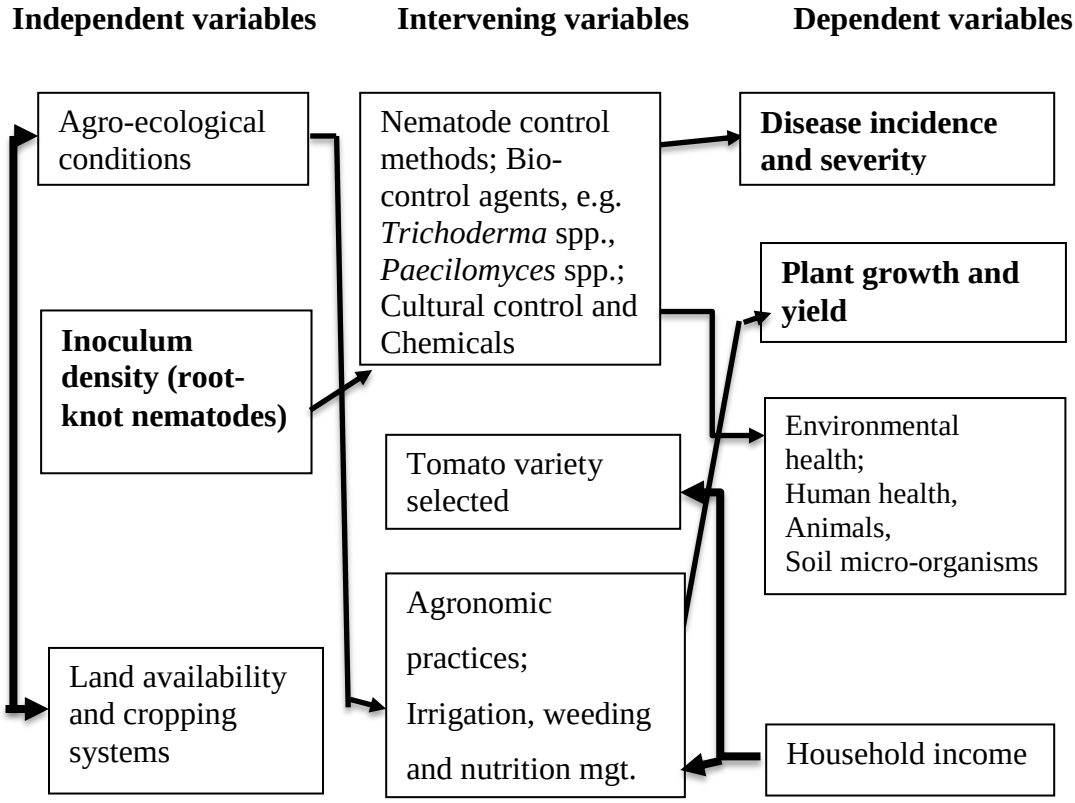
1. There are fungal organisms associated with tomato roots and Root knot nematodes egg masses.
2. Fungi associated with tomato and RKN egg masses significantly suppress root-knot nematodes survival *in vitro*.
3. Fungi antagonistic to RKN in vitro significantly suppress galling and promote growth and yield of tomatoes under green house and field conditions.

1.6 Significance of the study

The findings of this study and the knowledge generated are useful to tomato farmers in Kirinyaga County, other parts of Kenya and the world where root knot nematodes are a problem and the identified biocontrol agents can be applied. This study reinforces available information on the important status of root-knot nematodes as a constraint to tomato production in the region. The study has demonstrated the prevalence of fungi with potential antagonistic capacity against RKN. The findings identified effective antagonistic fungi that can be commercialised to contribute to sustainable management of root knot nematodes. The isolates contribute to the options available to small scale tomato farmers in their search for an inexpensive and environmental friendly nematode control strategy. In the end, it could lead to a healthy soil ecosystem and better yields. Overall, tomato growing farmers will achieve improved health, better income and livelihoods.

1.7 Conceptual framework

The following diagram shows the variables involved in the study and arrows showing the relationship among the different variables. It depicts the direction by which the research was undertaken.



CHAPTER 2: LITERATURE REVIEW

2.1 Tomato production and their economic importance in Kenya

Tomato (*Solanum lycopersicum* L.) is among the widely cultivated local market vegetables in Kenya. It has a critical role in revenue generation, foreign currency earning and creating job opportunities (Singh *et al*, 2017). In Kenya, tomato is mainly grown in open field and greenhouses (Nyamwamu, 2016) Kirinyaga County is the second leading tomato growers (10.2%) after Kajiado (12%) as noted in Table 2.1.

Table 2.1: Tomato production in selected counties in Kenya.

County	**Area (Ha)**	**Volume (Mt)**	**Value (KES)**	**% of Total**
Kajiado	3,024	71,250	2,379,680,250	12.0
Kirinyaga	2,460	60,587	2,037,800,000	10.2
Narok	2,420	54,082	1,886,227,500	9.5
Machakos	4,075	56,225	1,328,475,000	6.7
Kiambu	769	24,499	1,249,126,000	6.3
Taita Taveta	783	28,610	1,238,650,000	6.2
Makueni	931	27,675	941,600,000	4.7
Homabay	1,541	12,104	743,706,000	3.7
Lamu	491	16,242	693,153,000	3.5
Kisumu	536	19,030	592,650,000	3.0
Trans Nzoia	441	14,633	518,266,000	2.6
Kitui	735	13,588	459,685,000	2.3
Murang'a	1,315	9,250	448,946,300	2.3
Bungoma	564	11,129	442,570,000	2.2
Siaya	628	9,523	431,532,500	2.2
Laikipia	321	10,999	376,500,000	1.9
Bomet	550	9,849	320,578,000	1.6
Kwale	420	6,966	319,660,000	1.6
Meru	498	9,702	316,985,000	1.6
Nyeri	356	11,348	299,950,768	1.5
Others	5,405	97,167	2,877,810,938	14.5
Total	**28,263**	**574,458**	**19,903,552,256**	**100.0**

Source: (HCDA 2018)

2.1.1 Nutritional and medicinal benefits of tomato

The key nutritive ingredient in the tomato fruit is lycopene and high vitamin C and A. Vitamin C acts as an antioxidant that reduces the risk of arteriosclerosis, heart

disorders and some forms of cancer (Benton, 2008). Lycopene (anti-oxidant) helps in lowering the occurrence of heart disorders, prostate and lung cancer (Heber and Lu, 2002). Tomato can be used in various forms including fresh in salads, cooking foods and processed to ketch up, pickles and sauce.

2.1.2 Agro ecological requirements

Tomato is adapted to different climatic conditions, but does well in areas with relatively warm conditions. Tomatoes thrive best in deep, well-drained sandy loam soil that is abit acidic with a pH of 5.5 to 6.8 (Tilahun, 2013). Extreme pH can cause nutrients deficiencies. The soil should have good water holding capacity and aeration. The optimum temperatures for tomato growth ranges from 15 to 25 ^{0}C. The tomato plants usually adapt to a set of temperatures, but the tissues get damaged at temperatures below 10°C and above 38°C (Naika *et al*., 2005). Temperatures below 21°C can cause fruit abortion. Low temperatures also cause delayed formation of colour and ripening.

2.2 Challenges to tomato production

Many challenges have been reported to reduce tomato production. The tomato crop is confronted by biotic elements including pests and diseases, shortage of improved seeds, and abiotic factors like drought, inadequate input supply and low soil fertility (Gemechis *et al*., 2012; Anang *et al*., 2013). Pests and diseases are the major drawbacks facing the tomato industry (James *et al*., 2010). The major tomato diseases include Fusarium wilt (*Fusarium oxysporum*), early blight (*Alternaria solani*), bacterial wilt (*Ralstonia solanacearum*), late blight (*Phytophthora infestans*), leaf spots and tomato mosaic virus. The major pests affecting tomatoes include whiteflies (*Bemisia tabaci*),

spidermites (*Tetraynchus* spp.), nematodes notably the root-knot nematodes (*Meloidogyne* spp.), aphids (*Aphis gossypii*), thrips (*Frankliniella occidentallis*, *F. schultzei*), and leaf miners (*Tuta absoluta*) (Waiganjo *et al*., 2008; Masinde *et al*., 2011). Physiological disorders such as sun burn, scald and cracking also influence crop productivity.

Tomato production is also severely affected by water shortages. farmers are also negatively affected due to poor bargaining power in the market for their produce (Tshiala and Olwoch, 2010). The use of unadapted species, post-harvest losses and inappropriate cultural practices have been rated as major pitfalls in tomato production (Ogbomo, 2011).

Some other factors that may influence tomato productivity include accessibility to outreach services and inaccessible lending markets (Wachira, 2012).

2.2.1 Plant parasitic nematode infesting tomatoes

Plant parasitic nematodes (PPN) are minute roundworms that thrive in the soil and attack the roots of plants. These nematodes include: root-knot nematodes (*Meloidogyne* spp.), spiral nematodes (*Helicotylenchus* spp. and *Scutellonema* spp.), lance nematodes (*Haplolaimus* spp.), lesion nematodes (*Pratylenchus* spp.), reniform nematodes (*Rotylenchus* spp.), (*Tylenchulus* spp.) and dagger nematodes (*Xiphinema* spp.). *Meloidogyne* species are the most wide-spread and the most destructive genus of the plant parasitic nematodes (Jones *et al.*, 2013).

Meloidogyne species are polyphagous PPN with sexual dimorphism where the males are vermiform and active, while the females are pyriform and sedentary (Tariq, 2008). The mature females are 0.5-1.5 mm in length and 0.33-0.7 mm in width (Beije *et*

al., 1984). This genus alone comprised of more than 100 distinct species by January 2015.

Vegetables are affected by four main root knot species; the *Meloidogyne arenaria, M. hapla, M. incognita* and *M. javanica*. The *Meloidogyne* species can cause severe injuries to a broad spectrum of crops, and more so to vegetables, leading to great yield losses in tropics and sub-tropical regions (Sikora and Fernandez, 2005; Sahebani and Hadavi, 2008). In Kenya, losses have been attributed to *M. incognita* and *M. javanica* (CAB1 2002b).

Meloidogyne species are reported to cause damage on vegetables including tomato, African nightshade and amaranths in Kenya (Nchore *et al*., 2013) with the affected plants left unacceptable for export. *Meloidogyne* spp. is highly destructive and has wide distribution making it a serious pathogen of African leafy vegetables (Nchore *et al*., 2012b). The root-knot nematodes are reported to cause root knots (galls), impaired roots, yellowing and withering of plants that results to low biomass weight in black nightshade, cowpea, amaranths and jute mallow (Linguya *et al*., 2015). In addition, farmers' knowledge on the presence and the management of the nematodes remains quite low (Maina *et al.*, 2011). *Meloidogyne* spp. causes damage to root which facilitates entry by other disease causing micro-organisms. The disease complexes (synergy) formed with plant pathogenic fungi and bacteria increase damage relative to a single pathogen attack hence increasing the yield loss (Rivera and Aballay, 2008).

In tomato, an infected crop produces fewer fruits and the life span of the crop is reduced due to early senescence caused by insufficient water and nutrient uptake.

Meloidogyne spp. alone can cause a loss of 30-100% yield loss in tomato crop (Olabiyi, 2008).

2.2.2 The life cycle of root-knot nematodes

The *Meloidogyne* species take a short time with its life stages from eggs, four juvenile stages (J1-J4) and the adult ranging from six to eight weeks (Pakeerathan et al., 2009). The mature females can lay 1000-3000 eggs. The eggs develop into juvenile stage 1 (J1) within the egg membrane before moulting to form the J2. The J2 move through the soil towards the root tips, penetrate and migrate to the zone of elongation where they feed and then become sedentary (Bird, 1974). The J2 undergo the second and third moult resulting into J3 and J4, respectively. The J4 develops into adult (female, male). The male leaves the root system and become non-parasitic in the soil while the female lays eggs for the next generation. The lifecycle may be completed in about 25 days depending on host, climatic conditions and nematode species. For instance, on crops such as tomato, with a life cycle of approximately four months to maturity, the RKN will have about four generations which should be considered in management options (Luambano, 2010).

Root-knot nematodes are spread from one place to another through infested seedlings, farm implements and irrigation water and farm workers. The RKN problem has been noted to be more devastating on light, sandy soils and areas irrigated through furrow methods. The intensity of *Verticillium* and *Fusarium* wilt diseases is also increased by attack of plants by nematodes (Noling, 2014). Root knot nematode disease development is favoured by certain conditions including warm climate where for instance *Meloidogyne hapla* is bound to moderately warm soils with a standard

temperature scale of 15 to 25^{o}C; while species such as *M. incognita*, *M. arenaria* and *M. javanica* thrive well at 25 to 30^{o}C. At extreme temperatures' beyond 40^{o}C and below 5^{o}C, the development of the *Meloidogyne* species is hindered (Zhang and Schmitt, 1994). The practice of cultivating crops prone to nematodes continuously in the field, weeds in and around the fields and infected volunteer plants also favours disease development.

2.2.3 Symptoms of Root-knot nematodes

The distinguishing signs of the *Meloidogyne* spp. usually occur on the below-ground portions of the plants. Root knot galls grow on infected roots and this make them bigger in diameter than healthy roots. Infected roots exhibit a rough and clubbed appearance when several infections occur. Some species of the nematode cause roots to grow into a bushy root system and remain smaller showing rotting and sometimes death late in the season.

Plate 2.1: Below-ground symptoms of root-knot nematodes on tomato (Credit: Scot C. Nelson, plant pathologist)

Above-ground symptoms are often mistaken for nutrient deficiency, reduced growth and incipient wilting associated with fewer, small and pale green leaves. The infected plants produce few flowers and fruits that are poor in quality. Infected plants usually continue thriving and they are rarely killed before maturity, except when they occur in complexes with other disease causing pathogens like *Fusarium* spp. (Abawi and Barker, 1984; Suleman *et al.,* 1997).

2.3 Managing plant parasitic nematodes

Several techniques have been applied against plant parasitic nematodes but with varying success. These include synthetic nematicides, cultural (fallowing, cover copping, crop rotation and soil amendments), bio-control agents and integration with resistant varieties (Bridge 1996; Coyne *et al.,* 2009).

2.3.1 Chemical control methods

This involves the use of inorganic products to manage *Meloidogyne* spp. in the affected soils. Chemical nematicides have been the most effective techniques of eradicating *Meloidogyne* spp. in many farms. However, some have been restricted in use in many regions of the world, for example, methyl bromide and Aldicarb (Temik) (Onkendi et al., 2014). These products are able to bring down the number of *Meloidogyne* spp. in the soil; however, they are not effective once symptoms have developed (Sirias, 2011). Metham sodium is one of the nematicide proposed for use as an alternative to methyl bromide.

Nematicides are usually grouped as fumigants or non-fumigants. However, use of chemical nematicides has several drawbacks due to their toxic nature to man and

animals, pollution to environment and also high cost limiting their use to large scale farming. Plant parasitic nematodes can develop resistance to the chemicals. In addition, many small growers (majority of tomato Kenyan farmers) are unable to purchase nematicides because of the cost.

In the current study, Velum prime® nematicides was used as positive control. The active ingredient in Velum prime®, fluopyrum, works by affecting the mitochondrial respiratory system of nematodes. It hence disrupts energy synthesis which in turn immobilises the nematodes and eventually result to death.

2.3.2 Cultural control methods

These practises entail use of clean propagation materials, tolerant/resistant varieties, rotating susceptible and non-host crops, use of clean farm implements and intercropping. Most of these practices have been used to control *Meloidogyne* spp. with success in various parts of Africa. These techniques are favourable to small scale farmers as they are cheaper but they are not fully effective and should be applied in integration with other control techniques.

Crop rotation aims at reducing nematode densities for production of a less susceptible crop in the next seasons. Crop rotation is however constrained by the presence of several nematode species with very broad host ranges. A further challenge in small-scale production is the increased human population leading to reduction in available land for agricultural production.

Resistant cultivars are plants that permit nematode access and varying level of parasitism but not reproduction (Hunt et al., 2005). Resistant crops are able to limit *Meloidogyne* spp. multiplication thus providing a better alternative in management of

the nematodes. Root-knot nematodes resistant tomato cultivars including Sandokan and Assila have Mi-gene introduced which works against *M. arenaria, M. incognita* and *M. javanica* but not *M. hapla* (Sikora and Fernandez, 2005). Resistant cultivars are compatible with several other management strategies and are environmentally friendly hence providing a viable means of controlling RKN (Singh and Khurma, 2007).

2.3.3 Physical methods

These techniques usually aim at suppressing the nematodes present in the soil before planting and can be used in integrated management strategies to effectively control *Meloidogyne* spp. They include heat treatment and solarisation (Ioannou, 2001). Nico *et al*., (2003) noted that solarisation of soils in the nursery for three weeks resulted in reduced egg infectivity.

2.3.4 Biological control methods

Biological methods involve using living organisms to control *Meloidogyne* spp. Some biological products have shown significant activity against nematodes. Some of the microorganisms in these products are *Pochonia chlamydosporia (*Atkins *et al*., 2003), *Pasteuria penetrans*, *Bacillus firmus*, *Purpureocillium* and *Trichoderma* spp. (Wilson and Jackson, 2013). Bacteria, particularly *Pseudomonas aeruginosa* was reported to have an impact on tomato growth and reduced galling (Shankar *et al*., 2011). Nematophagous fungi provide an alternative in control of root-knot nematodes as an antagonist traps and endoparasites (Pendse, 2013). Most of these microorganisms' act through attachment to the nematode cuticle or parasitizing female eggs, subsequently eliminate the nematodes (Kariuki and Dickson, 2007). Endophytic fungi including

Fusarium oxysporum (FO162) are reported to influence resistance in crops like tomato against *Meloidogyne* spp. (Walters, 2009).

2.3.5 Nematophagous fungi

This is a group of various fungi known to invade and infest nematodes for utilization of nutritional components. The majority of nematophagous fungi are facultative parasites while some are obligate parasites of nematodes (Hallmann *et al.*, 2009). The mode of action against nematodes has been used to classify nematophagous fungi into four groups: nematode-trapping (= predacious or predatory fungi), endoparasitic, egg- and female-parasitic and toxin-producing fungi (Liu *et al*., 2009). The endophytes thrive within plant's tissue without adversely affecting the host crop and older plants may hold more endophytes than younger ones (Kogel *et al*., 2006). These fungi boost the growth of plants while protecting the host from pathogens and pests (Akello *et al*., 2007; Haggag, 2010). They are also important due to their ease of application as seed treatment or on transplants thus reducing the amount of inoculum and labour required for field application (Nzanza *et al*., 2011). Among the endophytes isolated frequently tomato roots, *Fusarium oxysporum* was found to be the most common of the non-pathogenic isolates that reduce *Meloidogyne* populations without severely affecting plant health (Hallmann and Sikora, 1994a, b and c).

Purpureocillium lilacinum (Lopez-Lima *et al*., 2014) formerly known as *Paecilomyces lilacinus* is a saprophytic soil fungus that principally infects and assimilates eggs of root knot (*Meloidogyne* spp.) and cyst nematodes (*Globodera* sp. and *Heterodera* sp.). *Paecilomyces lilacinus* is a widely tested bio-control agent against plant parasitizing nematodes (Atkins *et al*., 2003). It has been shown to occur widely in

the tropics and sub-tropics and present in almost all productive soils (Brand *et al*., 2010). The fungus is a lilac to purple coloured soil hyphomycete, producing smooth to rough conidia endogenously from small groups of unclamped phialides borne on conidiophores. The vegetative hyphae are branched and septate. *Paecilomyces lilacinus* can be found naturally in soils, egg clusters contained in gelatinous egg masses of RKN and cysts of *Globodera* sp and *Heterodera* sp. The damage on nematodes occurs through reduction in egg hatch and the number of root galls and egg masses may also be reduced or suppressed in some instances. Giant cell formation in host plant tissue is inhibited (Cabanillas *et al*., 1988).

Pochonia chlamydosporia var. *chlamydosporia,* a nematophagous agent, has been studied widely as a bio-control agent against plant parasitic nematode. It is one of the effective bio-control agents for nematodes such as *Globodera, Heterodera, Meloidogyne*, *Nacobbus* and just recently *Rotylenchulus* (Manzanilla-Lopez *et al*., 2013). *Pochonia chlamydosporia* is reported to act only through egg parasitism while *Aspergillus terreus* filtrate can suppress egg hatch and kill J2, but not parasitizing eggs. (Singh and Mathur, 2010).

Trichoderma species are asexually producing fungi occurring in most soil and root ecosystems and are recognized as potential bio-control agents (Kamala and Indira, 2012). Fast growth, ability to modify the rhizosphere and production of numerous spores and antibiotics are attributes that make most species highly successful. *Trichoderma harzianum* BI successfully infests nematode eggs and juveniles and significantly reduce *M. javanica* under greenhouse environment (Sahebani and Hadavi, 2008). Induced systemic resistance, plant growth promotion, antibiosis, mycoparasitism

and cell wall degenerating enzymes like cellulose and chitinase are the various mechanisms used by *T. harzianum* to suppress *Meloidogyne* spp. (Harman *et al*., 2004).

Arthrobotrys oligospora, the most widespread nematode capturing fungus that thrives mostly as a saprophyte. *A. oligospora* forms a complex three spatial matrix, only in the presence of nematodes, which trap the nematodes (Yang *et al*., 2011). Trapping involves adhering to the cuticle of nematode, penetrating and immobilizing it. *Arthrobotrys oligospora* produces two pathogenicity factors; a carbohydrate binding protein (lectin) and an extracellular serine protease (Yang *et al*., 2007). In tomato crops, *A. oligospora* was found to highly reduce the number of galls, egg masses and egg counts per tomato plant infected by *M. javanica* (Bakr *et al*., 2014).

Aspergillus spp. is a group of fungi found in the rhizosphere of most crops and have demonstrated ability to produce antagonistic metabolites that are lethal to nematodes (Jin *et al*., 2019). The different species of *Aspergillus* produce different toxins; *A. terreus* releases citrinin, *A. fumigatus* produce kojic acid while *A. flavus* produce aflatoxin. *Aspergillus* species are reported to hamper gall formation and egg mass production per root system and also promote plant growth (Zareen *et al*., 2001).

Acremonium strictum is a saprophyte found in the rhizosphere of crops and is reported to be an egg parasite of plant parasitic nematodes. It acts by egg parasitism and toxin production (Singh and Mathur, 2010). Hence the bio-control of root-knot nematodes provides a viable alternative to use of nematicides since it's effective and environmentally friendly.

The current study therefore aimed to improve tomato production by managing the root knot nematode using local bio-control agents. This study specifically focused

on isolation and identification of fungal antagonists associated with tomato and *Meloidogyne* species, evaluating the efficacy of the fungal isolates against eggs and juveniles of root-knot nematodes *in vitro*, under greenhouse and field conditions and evaluating the effects of the fungal isolates on growth and yield of tomatoes.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The research was carried out in Kimbimbi area, Mwea, Kirinyaga County (Figure 3.1). Mwea is a semi-arid region with an altitude of 1100 meters above sea level and rainfall ranges from 800-2200 mm annually. It is located at 0°36'8'' S 37º21'58'' E. The annual temperature ranges between 15.6 - 28.6°C. The main agro-ecological zone in Mwea division is the Low Midland Zone (LMZ) and the climate enables two short cropping seasons. Most people in Mwea practise agriculture through production of crops such as rice, tomatoes, maize, French beans, onions and beans, which are cultivated for commercial purposes. Tomatoes in this area are produced throughout the year since farmers use furrow irrigation.

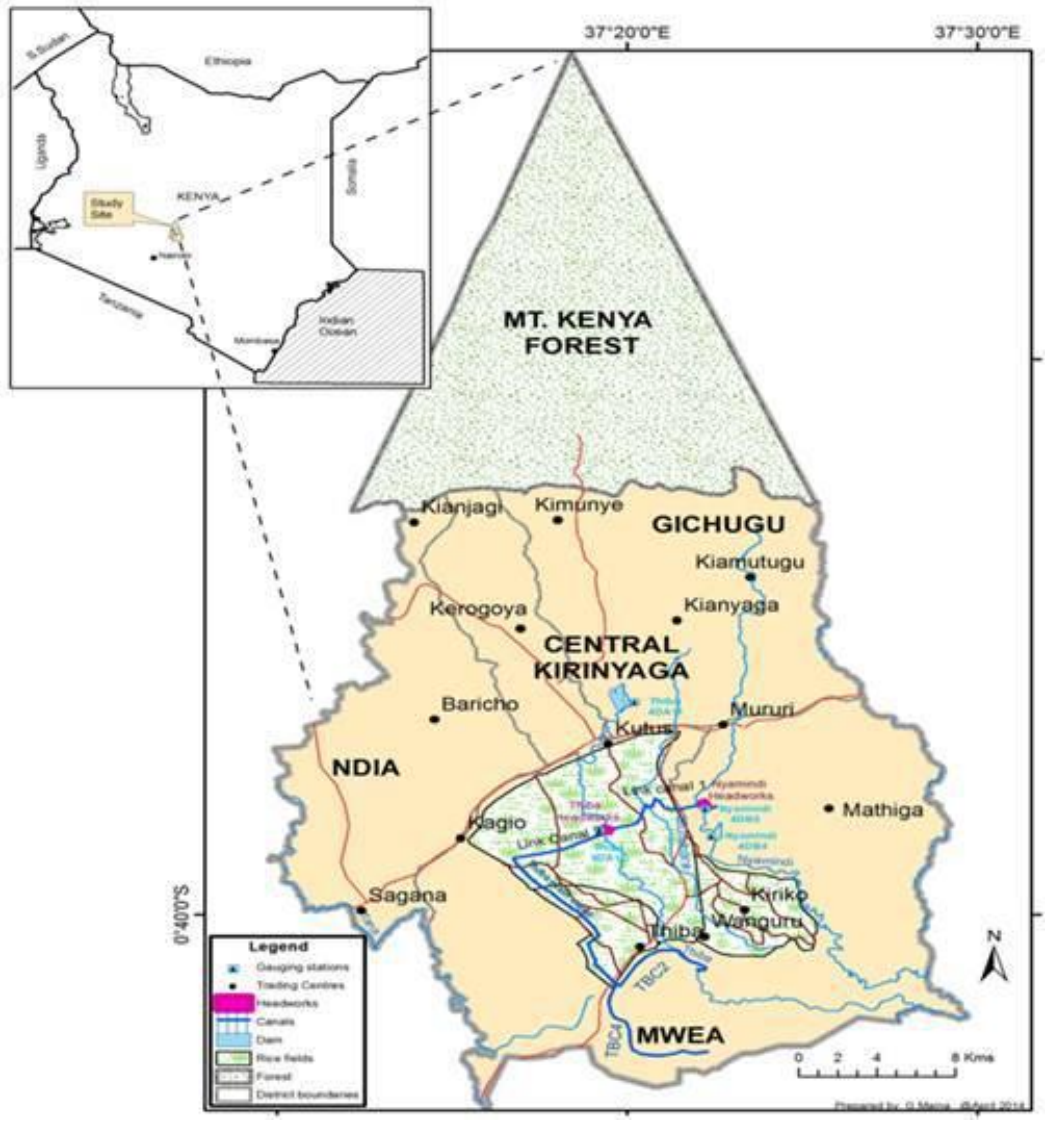


Figure 3.1 : Map of Kenya showing the location of Mwea in Kirinyaga County. Source: Serede *et al.* (2015)

3.2 Isolation and identification of fungal antagonists associated with tomato roots and root-knot nematodes eggs

3.2.1 Sample collection

Samples of healthy and heavily galled tomato roots were sampled from tomato farms through purposeful sampling in Mwea, Kirinyaga County. Representative samples (10) of tomato roots from each farm of approximately ½ acre were selected randomly in

a zigzag pattern and dug out softly using a shovel up to 30 cm below the ground. The soil attached to the roots was gently removed. The tomato root samples were then put in sample bags labelled and placed in a transportation cool box and taken to the laboratory for testing.

3.2.2 Isolation of fungal isolates from healthy tomato roots

The healthy tomato roots (presumed to have potential antagonistic fungi) sampled from each site were thoroughly but gently washed under tap water. The plant roots were then made sterile under a laminar airflow hood by dipping them in 0.5 % sodium hypochlorite (NaOCl) for 180 seconds after which they were rinsed out twice in purified water and air dried on sterile blotting paper according to Dabat *et al.* (2008).

After drying, the roots were chopped into 1 cm cubes and placed evenly on the surface of Potato Dextrose Agar (PDA) medium in the Petri plates in which 150 mg/l streptomycin was added to suppress bacterial growth. The plates were sealed with parafilm to avoid desiccation and contamination. The plates were incubated at 25°C for 7 days to promote fungal growth and sporulation. The fungal cultures were then sub-cultured onto fresh PDA medium to obtain pure cultures and observed after every three days for a period of two weeks. The final pure cultures were preserved on PDA slants at 4ºC in the refrigerator until further use.

3.2.3 Isolation of fungal isolates from egg masses

The egg masses were handpicked using forceps from the galled roots under a dissecting microscope and placed aseptically in Petri dishes containing PDA amended with Streptomycin. The Petri dishes were then incubated at 25 ^{o} C for 7 days. Pure

fungal cultures were obtained and used for bioassay and others maintained on PDA slants.

The various fungal isolates were identified in reference to the morphological features according to identification key (Watanabe, 2010). The selected isolates for *in vivo* studies were identified to the species level using molecular techniques at Kenya Plant Health Inspectorate service (KEPHIS) laboratories.

3.3 Evaluation of the efficacy of fungal isolates against *Meloidogyne* spp. *in vitro*

3.3.1 Preparation of root-knot nematodes (RKN) inoculum

Eggs were extracted from galled tomato roots through root maceration method as described by Coyne *et al*. (2007). The roots were properly washed under tap water and blotted dry using a paper towel. The roots were then cut into one-centimetre-long pieces. Roots were weighed and water added at a ratio of one gram of fresh root to 20 ml water and 1.5% NaOCl into a blender and blended for 15 secs at high speed (Hooper *et al*., 2005). The resultant suspension was sieved using 500 μm, 50 μm, 25 μm aperture sieves into a beaker and eggs enumerated using a dissecting microscope to estimate concentration per two millilitres. The eggs were then incubated for 7 days and freshly hatched J2 collected every 2 days after the third day of incubation. The number of second stage juveniles in the suspension was ascertained with the help of a counting dish under a compound microscope (Hussey and Barker, 1973). This concentrate was then adjusted to about 500 J2/ml for use in the laboratory and greenhouse trials.

3.3.2 Preparation of fungal inoculum

A conidial concentrate was prepared by flooding the surface of a 7 days old pure fungal culture with 5 ml of purified water to which 1% Tween 20 was added. The spores were dislodged with a sterile glass slide by gently scraping off the sporulated aerial mycelium. The resultant content was then filtered through three layers of cheese cloth. The density of the spores in the mixture was calculated using haemocytometer slide under a compound microscope and then sterile water was added to adjust it to 1×10^6 spores per ml (serial dilution) and used for the tests.

3.3.3 Effects of fungal isolates on root-knot nematodes eggs hatch rate

To test egg hatch rate, 1.0 ml of surface disinfected freshly prepared egg suspension (with sodium hypochlorite) containing 100 eggs was added in Petri plates containing the designated fungal isolate. Eggs were also placed on fungus free water agar plates as controls. The plates were sealed with parafilm. Each treatment comprised of three replications. Treatments were placed in a Completely Randomized Design (CRD) at room temperature of 23± 2°C (Javeed *et al*., 2016). After 3 and 7 days, eggs were examined under a dissecting microscope for hatching. The whole process was carried out in sterile environment.

Egg hatch rate was ascertained through enumerating all the eggs and J2 under a microscope and the rate computed according to the following formula:

Egg hatch rate $= \frac{100\times J2}{\text{Total no.of eggs}}$ (Al Ajrami, 2016).

3.3.4 Effect of fungal isolates on mortality of root-knot nematodes second stage juveniles

To assess the efficacy of the fungal isolates on juvenile mortality, 1ml containing 20 freshly hatched *Meloidogyne* sp. 2nd stage juveniles was transferred into 5 cm Petri dishes. The various fungal isolates were then considered as treatments. The plates were inoculated with fungal spore suspension and water as control then incubated at 23±2°C. After 7 and 14 days of incubation, juveniles were observed under a dissecting microscope. Juveniles were considered dead if they became rigid and did not show any response upon probing the tail with a mounting pin (Al Ajrami, 2016). Treatments were replicated three times and the percentage of death per each treatment calculated according to the following formula:

$$\text{Juvenile mortality} = \frac{100 \times \text{Dead J2}}{\text{Total no. of J2}}$$ (Al Ajrami, 2016).

3.4 Evaluation of the effectiveness of the selected fungal antagonists against root-knot nematodes under greenhouse and open field

3.4.1 Multiplication of the selected fungal antagonists

The five effective fungal isolates from the *in vitro* studies (*Trichoderma harzianum* 3, *Trichoderma harzianum* 1, *Purpureocillium lilacinum*, *Trichoderma afroharzianum* and *Trichoderma harzianum* 2) were propagated on sorghum grains. Sorghum grains substrate has been proved to be highly effective in multiplication of fungal isolates including *Trichoderma* species (Shahid, 2012; Kumar *et al*., 2014) The sorghum grains were immersed in water (containing 2% dextrose) in a ratio of 1:2 (sorghum: water) for 12 hours, surplus water was drained and the grains then put in polypropylene carrier and autoclaved at 121ºC at 15 psi for about 20 minutes. These

components were cooled to 25ºC and then infused with the 5mm cube of the 7 days old fungal mycelium (Cumagun and Moosavi, 2015).

The substrate in polypropylene bags was incubated for about 2 weeks with regular turning for uniform growth, air dried, blended and filtered through 80 and 50 μm mesh sieves concurrently to obtain the spore powder. The powdered form of sorghum grains containing the fungal isolates was incorporated in talc in the ratio 1:2 and dried at room temperature then mixed with carboxyl methyl cellulose (CMC) 5g/Kg of the product (Singh *et al*., 2016). The estimation of colony forming units (cfu) of the fungal species was achieved through serial dilution of the powder. These were then used in the green house and field experiments.

3.4.2 Greenhouse experiments

The greenhouse experiments were set up in Kenyatta University in the period of January-March 2019 and April-June 2019. Tomato seeds; Kilele F1 variety were raised on sterile soil mixture in a nursery bed for germination under greenhouse conditions. The potting medium was a mixture of sand and soil in the ratio 2:1. The potting medium was autoclaved at 121^{o}C for 20 min at 15 psi. Plastic bags measuring 40 cm in diameter and 20 cm depth were filled with sterile soil mixture and placed in the greenhouse. Three weeks old tomato seedlings were transplanted, one seedling per pot as shown in plate 3.1. The experiment was laid out in a Complete Randomized Design (CRD) with treatments being replicated 4 times. The treatments were;

T1- Untreated control (water only) inoculated with RKN,

T2- RKN + *Trichoderma harzianum* 1,

T3- RKN + *Trichoderma harzianum* 2,

T4- RKN + *Trichoderma harzianum* 3,

T5- RKN + *Purpureocillium lilacinum*,

T6- RKN + *Trichoderma afroharzianum*

T7- RKN + Bionematon® (*Paecilomyces lilacinus* 1.15%) (Positive check) and

T8- RKN + Velum prime® SC 500 (Fluopyram 500 g/L) (synthetic nematicides).

The five fungal isolates were selected based on the performance in the in vitro studies as well as their ease in growth and multiplication. The fungal isolates were applied immediately after transplanting at 1.0×10^6 spores/ml per pot. A plastic syringe was used to place juveniles into 3cm deep holes made around the root system. The plants were inoculated with a 1000 juveniles per pot. Proper agronomic practices were adhered to and experiment terminated at 90 days after transplanting.

Plate 3.1: Tomato plants after treatment application in the greenhouse

3.4.3 Data collection

Tomato growth parameters (shoot height, length of roots, root and dry shoot and root weights) were determined. Shoot height (cm) was estimated from the soil line to the shoot apex using a tape measure 30, 60 and 90 days after transplanting

corresponding to the crop phrenological stages (vegetative, flowering and maturity stages).

At termination, plants were lightly plucked up and roots separated from the shoots by cutting at the soil line. The roots were rinsed under running water, surface sterilized using 1.5% NaOCl and then rinsed in distilled water. The roots were then blotted dry using a paper towel and the length of roots was determined by use of a tape measure from the soil line to the longest root tip. The root and shoots were then dried at 60^{o}C in an oven until a steady weight was arrived at and their dry weight calculated as outlined by Arim *et al*., (2006).

The disease parameters were determined as follows; the juvenile distribution in the soil was estimated by soil sampling at 30, 60 and 90 days after inoculation. The juveniles were extracted from the soil through modified Bearman's technique as described by Coyne *et al*. (2007). For counting the nematodes, the extract was passed through a series of aperture sieves (500 μm, 50 μm, & 25 or 32 μm). The nematodes were back washed from the sieve and concentrated into 8-10 ml for counting under the microscope.

The galling and egg mass indices were ascertained by calculating the number of galls/egg masses and then scoring according to Quesenberry *et al*. (1989) using a scale of 0-5 where: 0 = no gall or egg mass; 1 = 1or 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100 and 5 = > 100 galls or egg masses per root system. To facilitate counting of egg masses the roots were stained for 15 minutes in an aqueous solution of Phloxine B stain (0.15 g/L water), which stains the gelatinous matrix pink-red increasing egg mass visibility (Holbrook *et al*., 1983).

3.4.4 Field experiment

An assessment of previous season crop and soil analysis for presence of root-knot nematodes was carried out to identify a field with high nematodes infestation. A two season experiment was conducted in January-April 2019 (season one) and May-August 2019 (season two) on separate fields. The experiment was laid out in a Randomized Complete Block Design (RCBD) with treatments replicated four times. The treatments were; Untreated control, *Trichoderma harzianum* 3, *Trichoderma harzianum* 1, *Purpureocillium lilacinum*, T5- *Trichoderma afroharzianum*, *Trichoderma harzianum* 2, Bionematon® (*Paecilomyces lilacinus* 1.15%) (Positive check) and Velum prime® SC 500 (Fluopyram 500 g/L) (synthetic nematicides). The plot size was 16 M^2 with 1M left between treatments and replicates

Four weeks old tomato seedlings raised in nursery beds were transplanted in the respective field and their growth monitored. Application of treatments was done immediately after transplanting as a drench and 2 subsequent applications at 30 days' interval.

3.4.5 Data collection

Soil sampling was carried out before transplanting tomato plants to determine the initial nematodes counts. Subsequent soil sampling at the interval of one month after transplanting and at the end of the trial to assess the population of root-knot nematodes in the soil was done. Data on plant height (cm) were recorded 30, 60 and 90 days after transplanting. Five plants were used as the sample size for determining the effect of fungal isolates on *Meloidogyne* spp. The numbers of galls/egg masses were counted per root system and galling index/ egg mass index scored on a 0-5 scale as described in

section 3.4.3 e.g. plate 3.2 Reproduction Factor (RF) was obtained from the ratio of final nematode population to initial population.

At termination, the tomato plants were plucked and roots separated from the shoots. The roots were rinsed softly using running water and dabbed dry using paper towel. Shoot heights were estimated from the bottom of the stem at the soil horizon to the highest node while the root lengths were measured from the soil horizon to the longest tap root. The root and shoots were then dried in the oven at 60^{o} C till an unwavering weight was attained (Arim *et al*. ,2006). The yield was recorded in terms of weight of marketable and non-marketable fruits/plot and converted to yield per hectare (Kg/ha).

A: Healthy tomato roots	B: Galled tomato roots

Plate 3.2: Healthy tomato roots GI=0 (A); Galled tomato roots GI=4 (B)

3.4.6 Data analysis

The data collected on egg hatch rate, mortality of juveniles, plant height, root length, dry shoot and root weight, J2 population in the soil, galling indices, egg mass indices, reproduction factor and yield was subjected to Analysis of variance (ANOVA) using SAS software (version 9.2). The separation of means was done using the Fisher's Least Significant Difference (LSD) at 5% level of significance.

CHAPTER 4: RESULTS

4.1 Identification of isolated fungi

Forty-five fungal isolates in total were recovered; 27 isolates from the tomato roots and 18 isolates from RKN eggs collected. The fungal isolates included *Trichoderma* spp. (38%) which was the most abundant followed by *Fusarium* spp. (33%) while the least abundant were *Penicillium* spp. (2%) and *Aspergillus* spp. (2%).

Trichoderma spp. and *Fusarium* spp. were obtained from both roots and egg masses, however, *Trichoderma* spp. were more frequent in RKN egg masses than in tomato roots while *Fusarium* spp. were more frequent in tomato roots than in RKN egg masses. *Purpureocillium* spp. and *Lasidiplodia* spp. were only obtained from the RKN eggs (Figure 4.2). *Penicillium* spp. and *Aspergillus* spp. on the other hand were only obtained from the tomato roots (Figure 4.1). A few isolates could not be identified due to lack of spore formation and were therefore classified as unidentified isolates (sterile mycelia).

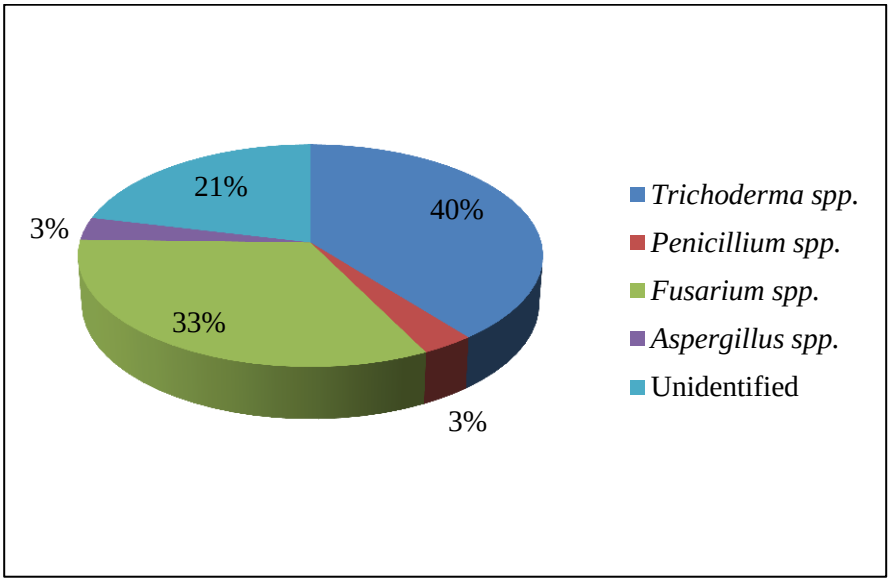


Figure 4.1: Proportion of fungal isolates from tomato roots

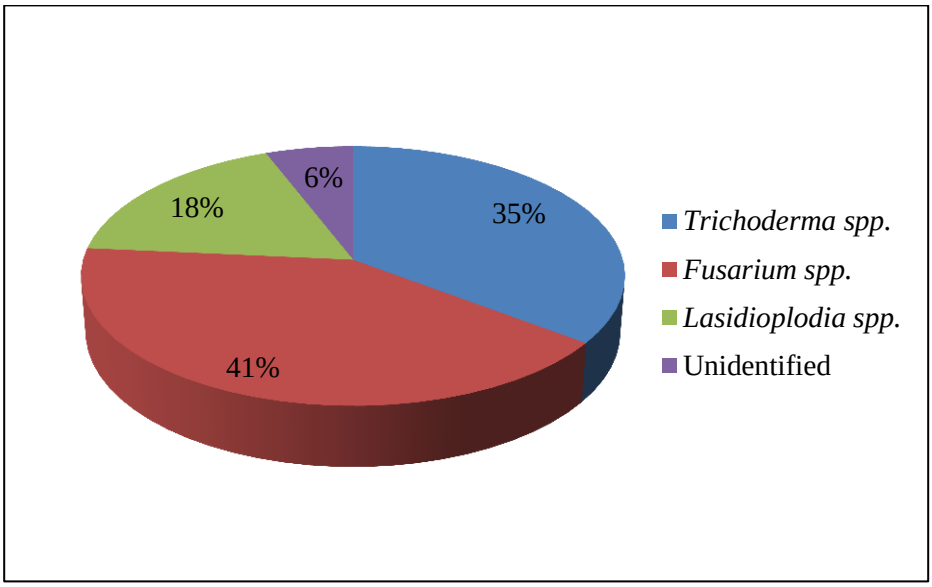


Figure 4.2: Proportion of fungal isolates from RKN Egg masses

4.1.1 Description of fungal isolates

4.1.1.1 *Trichoderma harzianum*

There were three isolates identified as *Trichoderma harzianum*. The *T. harzianum* strains differed in their morphology and growth rates.

On Potato Dextrose Agar (PDA), macroscopic observation showed rapid growth rate of *T. harzianum* 1 which appeared as a white colony with radial growth (Plate 4.1) that produced green conidia upon sporulation.

Trichoderma harzianum 2 and 3 also appeared as a white colony that formed 2-3 concentric rings (Plate 4.2 and 4.3) with green conidial production upon sporulation while the reverse side of the plates was pale. The spores were denser at the centre than towards the margins (Plate 4.3). These conidia were light green in colour while the phialides were flask shaped and arranged in a divergent form.

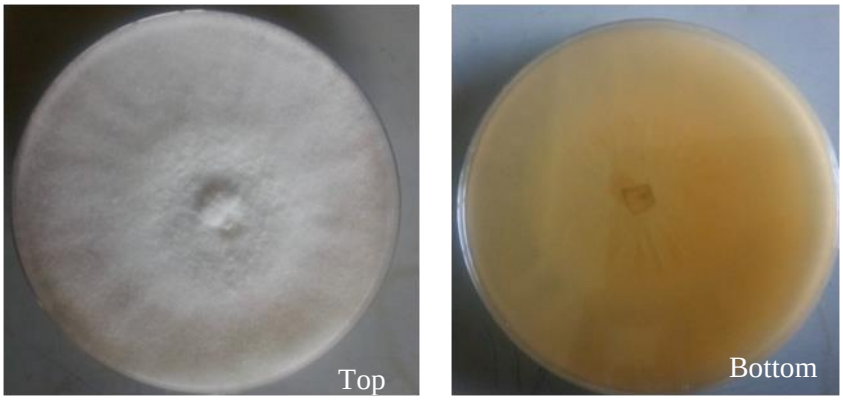


Plate 4.1: *Trichoderma harzianum* strain 1 (isolated from roots) colony growing on PDA

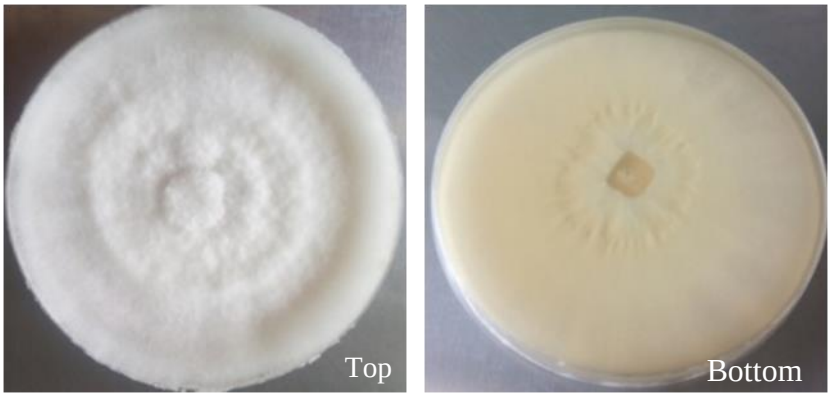


Plate 4.2: *Trichoderma harzianum* strain 2 (isolated from roots) colony growing on PDA

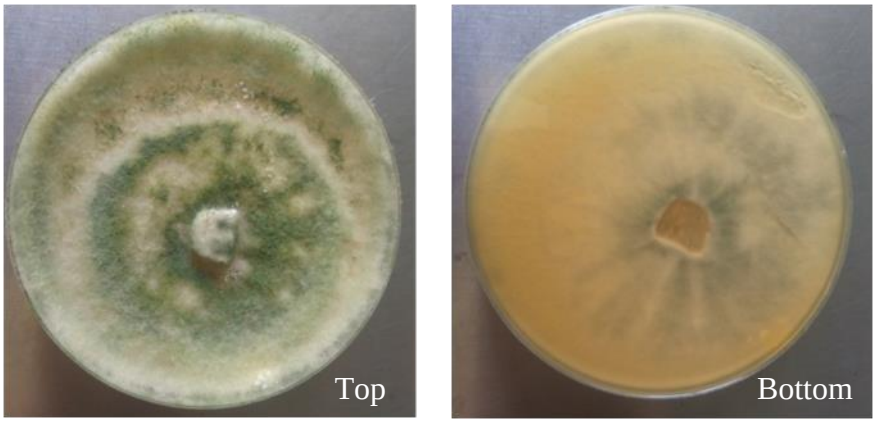


Plate 4.3: *Trichoderma harzianum* strain 3 (isolated from roots) colony growing on PDA

4.1.1.2 *Trichoderma afroharzianum*

Trichoderma afroharzianum had a rapid growth and the colony diameter after 5 days was full plate (9.0 cm). The colonies were white on PDA medium after three days

of initial plating and later developed yellowish green conidia which were distributed throughout the plate (Plate 4.4). The reverse side appeared pale. Conidia were abundant, one celled and ovoid while the conidiophores were extensively branched.

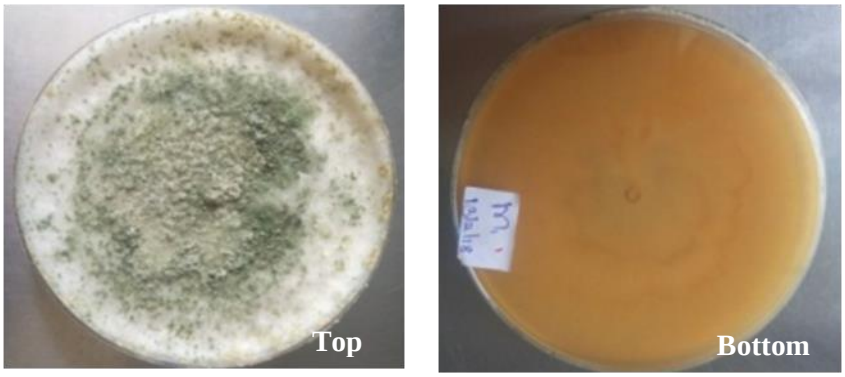


Plate 4.4: *Trichoderma afroharzianum* (isolated from RKN egg masses) colony on PDA

4.1.1.3 *Purpureocillium lilacinum*

Purpureocillium lilacinum on PDA showed a moderately fast growth with white colony at first then becoming vinaceous to violet/pink coloured while the reverse appeared in shades of purple (Plate 4.5). Conidiophores were verticillate with two to four phialides having a swollen basal portion tapering into a short distinct and slender neck. Conidia were smooth-walled to slightly roughened, hyaline to purple in mass and are produced in divergent chains.

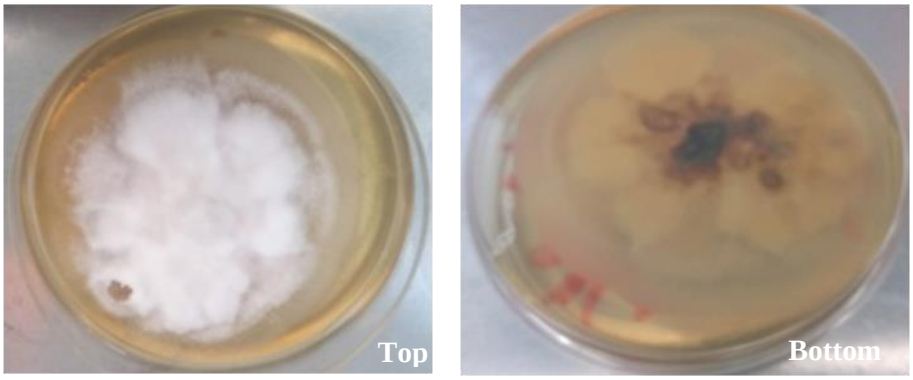


Plate 4.5: *Purpureocillium lilacinum* (isolated from RKN egg masses) colony growing on PDA

4.2 Efficacy of the fungal isolates on root-knot nematodes *in vitro* tests

4.2.1 Effects of fungal isolates on eggs hatch rate

The 19 fungal isolates inhibited egg hatching significantly ($P \leq 0.05$) when compared to the untreated control (Table 4.1). The egg hatch rate recorded after 7 days of exposure was significantly ($P \leq 0.05$) higher than that noted 3 days after exposure to the various fungal culture filtrates. *Trichoderma harzianum* 1 was the best in inhibiting egg hatching after 3 days while *Trichoderma harzianum* 2, performed best after 7 days. Six isolates of *Trichoderma* species (*T. harzianum* 1, *T. harzianum* 2, *Trichoderma*.sp.7, *Trichoderma* spp.6, *Trichoderma* spp.9 and *T. harzianum* 3) had higher inhibition rate compared to *Purpureocillium* spp. although these were not significantly ($P > 0.05$) different after 3 and 7 days of exposure to fungal isolates (Table 4.1). The highest hatching rates were recorded in the untreated control (68.33%) after 3 days and 69.33% after 7 days of exposure to the fungal isolates (Table 4.1).

Table 4.1: Mean egg hatching (%) of *Meloidogyne* spp., 3 and 7 days after exposure to fungal bio-control agents

Treatment	**Mean egg hatching (%)**	
	3 days	**7 days**
T. harzianum 1	21.00±5.03 e	22.67±4.80 de
T. harzianum 2	21.33±3.93 e	22.33±3.93 e
T. harzianum 3	28.00±6.56 cde	29.33±6.44 cde
P.lilacinum	30.67±4.70 bcde	32.67±3.71 bcde
T. afroharzianum	32.67±6.39 bcde	33.67±6.96 bcde
Trichoderma spp. 7	27.00±6.51 de	28.67±6.69 cde
Trichoderma spp. 6	30.00±7.00 bcde	31.67±7.27 bcde
Trichoderma spp. 9	32.00±8.02 bcde	32.67±8.21 bcde
Trichoderma spp. 3	31.33±6.94 bcde	33.67±6.96 bcde
Trichoderma spp. 8	33.00±9.07 bcde	34.67±9.60 bcde
Fusarium spp. 3	36.67±4.49 bcde	38.33±6.44 bcde
Fusarium spp. 1	38.00±2.31 bcde	39.00±2.08 bcde
Fusarium spp. 4	40.00±7.64 bcde	41.33±7.86 bcde
Fusarium spp. 9	40.00±6.66 bcde	41.67±7.27 bcd
Fusarium spp. 13	41.33±12.03 bcd	42.33±11.85 bc
Fusarium spp. 6	43.33±4.37 bcd	45.00±5.00 bc
Fusarium spp. 12	45.00±4.58 bcd	46.67±4.41 bc
Fusarium spp. 7	47.00±4.51 bc	48.67±4.33 b
Penicillium spp.	48.00±9.85 b	49.33±9.77 b
Untreated (control)	68.33±5.24 a	69.33±5.24 a
L.S.D	**19.07**	**19.31**
P	**0.005**	**0.007**

Data are means of three replicates (n=3). Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

4.2.2 Effects of fungal isolates on J2 mortality

The various fungal isolates caused a remarkable (P≤0.05) increase in juvenile (J2) mortality as opposed to the control (Table 4.2). The isolates were considered effective if they achieved 50% J2 mortality after the 7 days. The highest J2 mortality observed was 88.3% and 91% after 7 and 10 days of exposure respectively with *T. harzianum 2* followed by 86.67 % and 90.33% by the *Trichoderma* spp. 7 (Table 4.2).

The least J2 mortality was recorded in *Penicillium* spp. (45 and 45.67 %) after 7 and 10 days of exposure respectively and which was significantly (P≤0.05) different from the untreated control (1.67 and 2%) juvenile mortality (Table 4.2). Four of the *Trichoderma* species were noted to perform better in causing a higher mortality rate as compared to *Purpureocillium* spp. though they were not different statistically (P>0.05) (Table 4.2).

Table 4.2: Mean juvenile mortality rate (%), 7 and 10 days after exposure to various fungal isolates

Treatments	**Mean juvenile mortality (%)**	
	7 days	**10 days**
T. harzianum 1	75.00±15.00 abcde	80.33±12.25 abc
T. harzianum 2	88.33±6.01 a	91.00±4.73 a
T. harzianum 3	55.00±5.00 def	59.33±6.69 bcde
P. lilacinum	80.00±7.64 abcd	82.33±7.31 ab
T. afroharzianum	81.67±4.41 abcd	84.67±3.76 a
Trichoderma spp. 7	86.67±3.33 ab	90.33±3.76 a
Trichoderma spp. 6	63.33±9.28 bcdef	66.67±7.94 abcde
Trichoderma spp. 9	86.67±6.67 ab	89.33±5.37 a
Trichoderma spp. 3	65.00±5.77 abcdef	68.33±5.24 abcde
Trichoderma spp. 8	56.67±14.81 cdef	59.33±15.86 bcde
Fusarium spp. 3	80.00±2.89 abcd	84.67±5.39 a
Fusarium spp. 1	51.67±7.27 ef	53.00±7.37 de
Fusarium spp. 4	55.00±2.89 def	57.67±4.98 cde
Fusarium spp. 9	61.67±13.02 cdef	67.00±12.70 abcde
Fusarium spp. 13	45.00±7.64 f	46.33±6.98 e
Fusarium spp. 6	75.00±17.56 abcde	76.67±16.90 abcd
Fusarium spp. 12	45.00±10.41 f	47.00±10.02 e
Fusarium spp. 7	51.67±4.41 ef	53.67±3.48 de
Penicillium spp.	45.00±7.64 f	45.67±7.45 e
Untreated (control)	1.67±1.67 g	2.00±1.53 f
L.S.D	**25.19**	**24.44**
P	**0.0001**	**0.0001**

Each value is the mean of three replicates (n=3). Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

4.3 Effects of different fungal bio-control agents on the growth of tomato plants in greenhouse experiment 1

4.3.1 Effect of fungal bio-control agents on shoot height

Five fungal isolates (*Trichoderma harzianum* 1, 2 and 3, *T. afroharzianum* and *Purpureocillium lilacinum*) were tested for efficacy against root-knot nematodes and their effects on tomatoes with Bionematon® as positive check and untreated plots as control. Velum prime®, a synthetic nematicide was also used in the experiment.

There were highly significant ($P \leq 0.05$) differences in shoot height (cm) of treated tomato plants in comparison to the untreated control (Figure 4.3). The plants were noted to increase in height over time in all the treatments and the increase from 30-60 DAT was at higher rate (77-120%) compared to 60-90 DAT (14-18%). The synthetic nematicides (Velum prime®) recorded the highest shoot heights throughout the test period which were statistically ($P \leq 0.05$) higher than those recorded in all other BCAs and control. On the other hand, the untreated plants consistently recorded the shortest shoots over the test period and differed significantly ($P \leq 0.05$) from the treated plants (Figure 4.3).

At 30 DAT, no statistical ($P > 0.05$) differences were noted in shoot heights among the bio-control agents (BCAs). However, *Trichoderma harzianum* 1 treated plants had taller shoots that were not significantly ($P > 0.05$) different from those noted in Velum prime® treated plants (Figure 4.3). At 60 DAT, Bionematon® recorded taller shoots followed by *T. harzianum* 1 though they were not significantly different from shoot heights with *T. harzianum* 2, *P. lilacinum* and *T. harzianum* 3. *Trichoderma*

afroharzianum treated plants recorded significantly shorter shoots than the other BCAs (Figure 4.3).

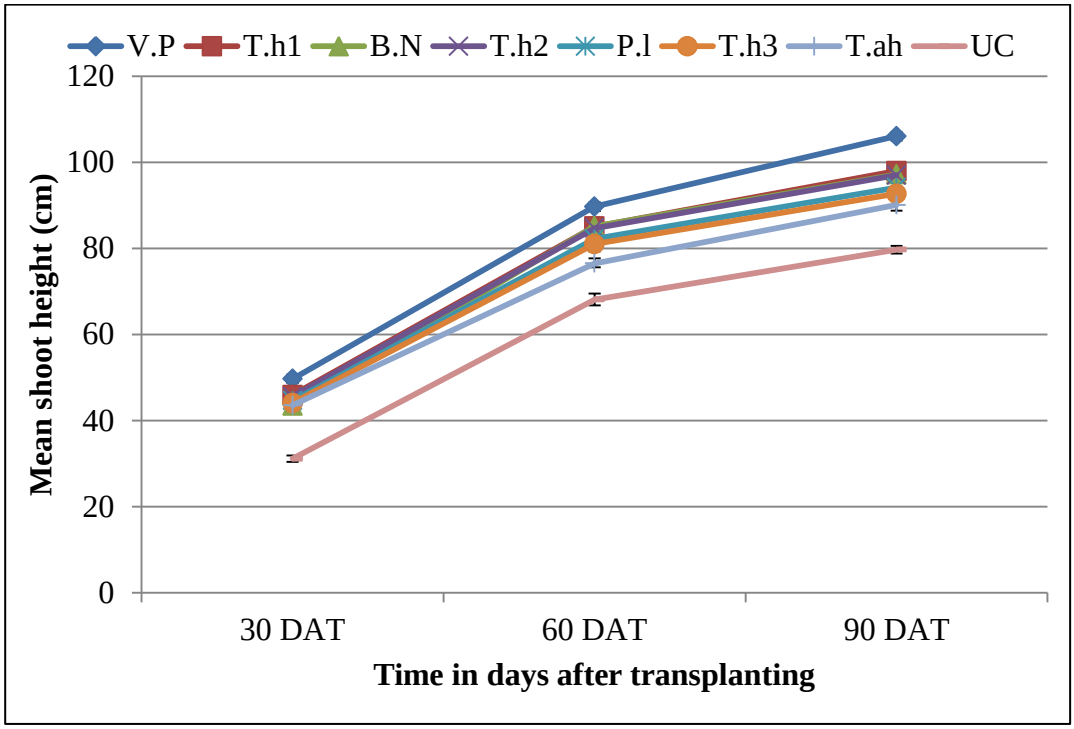


Figure 4.3: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the greenhouse experiment 1

Data are mean of 4 replicates. DAT=Days after transplanting, V. P= Velum prime®, T.h1=*Trichoderma harzianum* 1, B. N= Bionematon®, T.h2= *T. harzianum* 2, T.h3= *T. harzianum* 3, T. ah=*T. afroharzianum*, UC=Untreated control.

There were significant differences in shoot heights of treated plants at 90 DAT. Among the BCAs, *T. harzianum* 1 treated plants recorded significantly ($P \leq 0.05$) taller shoots than *P. lilacinum*, *T. harzianum* 3 and *T. afroharzianum* treated plants. However, the shoot heights did not differ from those noted with Bionematon® and *T. harzianum* 2. *Trichoderma afroharzianum* treated plants recorded shorter shoots and did not differ significantly ($P > 0.05$) from *T. harzianum* 3 treated plants (Figure 4.3).

Overall, *T. harzianum* 1 performed better in promoting plant growth with 23% increase in shoot height over the untreated control than Bionematon® (22%) and *P. lilacinum* (18%). Bionematon® treated plants performed significantly (P≤0.05) better than *P. lilacinum* in terms of shoot heights (Figure 4.3).

4.3.2 Effects of fungal bio-control agents on dry shoot and root weights (g) and root length (cm) of tomatoes

The dry weights of treated tomato plants were higher and significantly (P≤0.05) different from those recorded in untreated plants (Table 4.3). Tomato plants treated with Bionematon® had higher dry weight of shoots although not significantly (P>0.05) higher than those recorded in *T. harzianum* 1, *T. harzianum* 2 and *P. lilacinum* treated plants. The commercial *P. lilacinum* (Bionematon®) treated plants were not significantly (P>0.05) different from local isolate of *P. lilacinum* with regard to dry weight of shoots.

In terms of dry root weight, *T. harzianum* 2 treated plants recorded higher dry root weights among the BCAs but was not significantly (P>0.05) different from Bionematon® and *P. lilacinum* treated plants. The untreated plants recorded the lowest dry weights in both roots and shoots (Table 4.3).

The results showed highly significant (P≤0.05) differences in root length (cm) between the treated and untreated tomato plants (Table 4.3). The highest root length was recorded in Velum prime® treated plants although this was not significantly (P>0.05) different from root length recorded for Bionematon® and *T. harzianum* 1 treated plants. The root lengths recorded with *T. harzianum* 2 and *P. lilacinum* were slightly lower than that recorded in *T. harzianum 1* treated plants but not significantly different. Shorter

root length was recorded with *T. afroharzianum* which did not differ significantly (P>0.05) with *T. harzianum 3* (Table 4.3).

Table 4.3: Mean dry shoot and root weights (g) and root length (cm) of tomatoes under different treatments in the greenhouse (Expt. 1)

Treatments	**DSW**	**DRW**	**RL**
Velum prime®	8.43±0.15 a	5.48±0.32 a	18.81±0.27 a
T. harzianum 1	7.98±0.12 b	4.35±0.25 cd	18.19±0.23 a
Bionematon®	8.03±0.15 a	4.84±0.27 abc	18.01±0.20 ab
T. harzianum 2	7.94±0.16 bc	5.05±0.27 ab	17.28±0.37 bc
P. lilacinum	7.83±0.14 bc	4.67±0.23 bcd	17.14±0.38 bc
T. harzianum 3	7.53±0.10 cd	3.98±0.23 de	17.08±0.17 cd
T. afroharzianum	7.41±0.15 d	4.36±0.22 cd	16.24±0.28 d
Untreated control	6.21± 0.22 e	3.32±0.18 e	12.99±0.50 e
LS**D**	**0.42**	**0.97**	**0.88**
p	**0.0001**	**0.0001**	**0.0001**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.
DSW=Dry shoot weight, DRW=Dry root weight, RL= Root length.

4.3.3 Effects fungal bio-control agents on yield of tomatoes

The results showed significant (P≤0.05) differences in yield (Kg/plant) of tomatoes between the treated and untreated plants (Figure 4.4). The highest marketable yield was recorded in Velum prime® treated plants and higher than all the other treatments. Among the BCAs, *T. harzianum* 1 treated plants had higher yield which was not significantly (P>0.05) different from *T. harzianum* 2 and Bionematon® treated plants. *Trichoderma harzianum* 3 had lower yield compared to the other BCAs but differed statistically (P≤0.05) from untreated control (Figure 4.4).

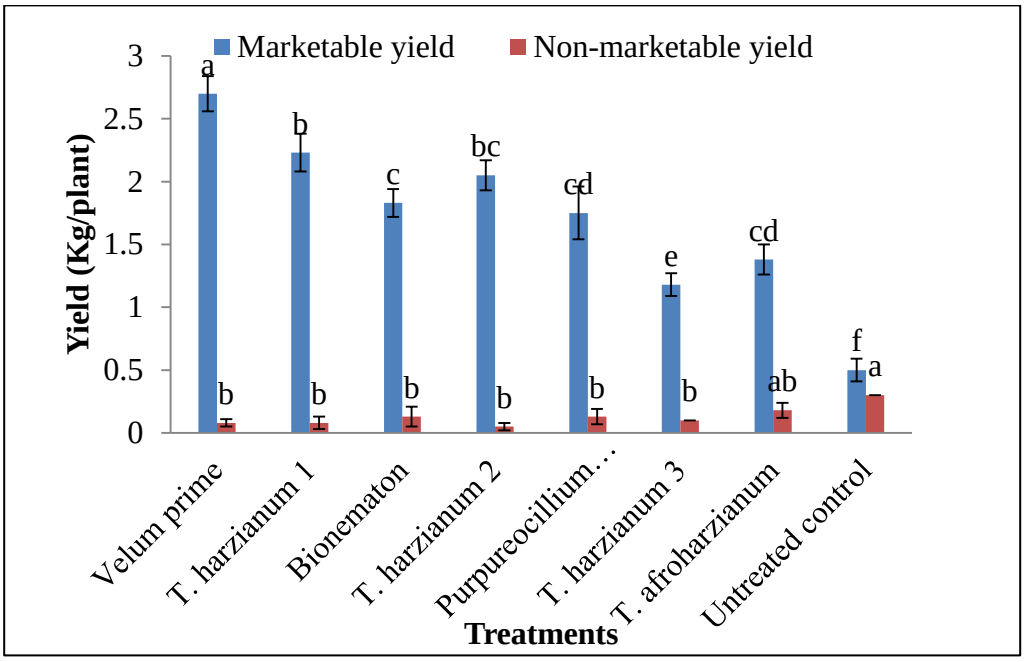


Figure 4.4: Mean yield of tomatoes in the greenhouse (Expt. 1)

Means followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. Data are mean ± SE of tomato fruits.

On further analysis a significant negative correlation (r=-0.79, P≤0.05) was established between the total yield of tomatoes and juvenile population at harvest (Figure 4.5).

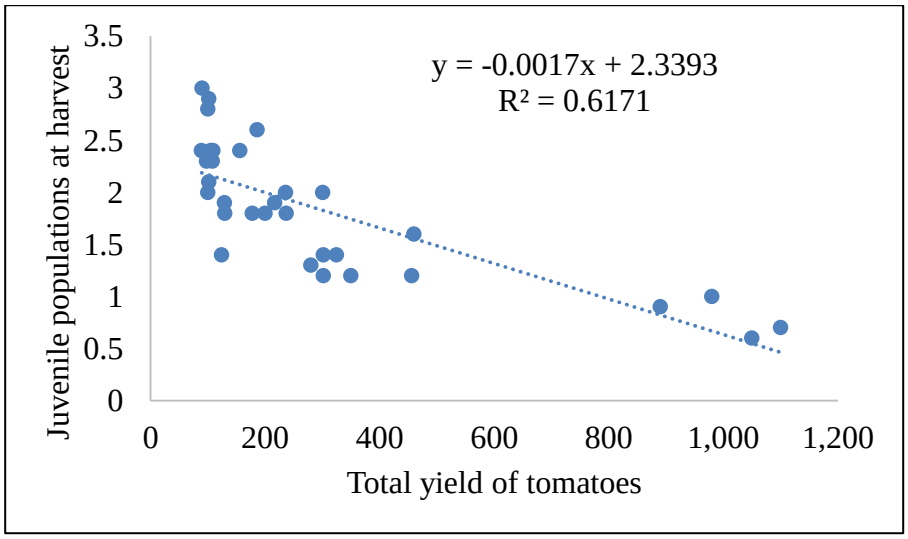


Figure 4.5: Relationship between yield of tomato and J2 population at harvest in greenhouse (Expt. 1)

4.4 Effects of fungal bio-control agents on the disease parameters of root knot nematode (*Meloidogyne* spp.) in greenhouse experiment 1.

4.4.1 Effects of fungal bio-control agents on second stage juvenile (J2) populations

The J2 population in the soil was observed to reduce over time in the treated pots while it increased in the untreated control (Table 4.4). At 30 days after inoculation (DAI), the least mean J2 population was recorded in Velum prime® treated pots which differed significantly ($P \leq 0.05$) from all the other treatments. Among the BCAs, *T. harzianum* 1 and *T. harzianum* 3 recorded a lower mean J2 population which did not differ significantly ($P > 0.05$) from the mean recorded with Bionematon®, *T. harzianum* 2 and *T. afroharzianum*. However, it differed significantly ($P \leq 0.05$) from *P. lilacinum* (Table 4.4).

At 60 DAI, Velum prime® treated pots still recorded lower mean j2 populations in the soil. The J2 populations were not significantly ($P \leq 0.05$) different from the means recorded with Bionematon®, *T. harzianum* 1, *T. harzianum* 2 and *P. lilacinum*. *Trichoderma afroharzianum* treated pots recorded higher mean J2 population and they differed significantly from Velum prime® treated pots. The J2 populations in *T. afroharzianum* treated pots, however, did not differ significantly from *T. harzianum* 3 treated pots (Table 4.4).

The least mean number of J2/200 cc of soil was recorded in Velum prime® treated pots followed by *T. harzianum* 1 treated pot. These were not significantly ($P > 0.05$) different from *T. harzianum* 2, *P. lilacinum* and Bionematon® at 90 DAI. *Trichoderma afroharzianum* was the least effective in reducing the mean number of J2 in the soil but differed significantly ($P \leq 0.05$) from the untreated control (Table 4.4).

Table 4.4 : Mean J2 populations in the soils treated with different treatments, 30, 60 and 90 days after inoculation in the greenhouse (Expt. 1)

Treatments	**Mean J2/200cc of soil**		
	30 DAI	**60 DAI**	**90 DAI**
Velum prime®	270.75±52.39 d	234.75±15.63 d	95.25±3.35 d
T. harzianum 1	496.25±39.89 c	312.50±41.13 cd	132.50±18.66 d
Bionematon®	590.00±28.58 bc	334.75±25.91 cd	199.50±41.41 bcd
T. harzianum 2	521.50±27.17 bc	336.25±34.57 cd	155.00±29.79 d
P. lilacinum	602.50±28.98 b	357.25±48.18 cd	183.25±52.41 cd
T. harzianum 3	496.25±34.45 c	431.50±71.47 bc	308.50±14.77 bc
T. afroharzianum	515.00±42.07 bc	514.50±86 b	319.00±83.45 b
Untreated control	815.50±19.03 a	880.00±37.19 a	1005.10±45.55 a
L.S.D	**103.45**	**147.79**	**126.24**
P	**0.0001**	**0.0001**	**0.0001**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DAI: Days after inoculation.

On further analysis, a positive correlation (r=0.74, P≤0.05) was established between the number of galls per root system of the tomato plants and the J2 populations in the soil (Figure 4.6).

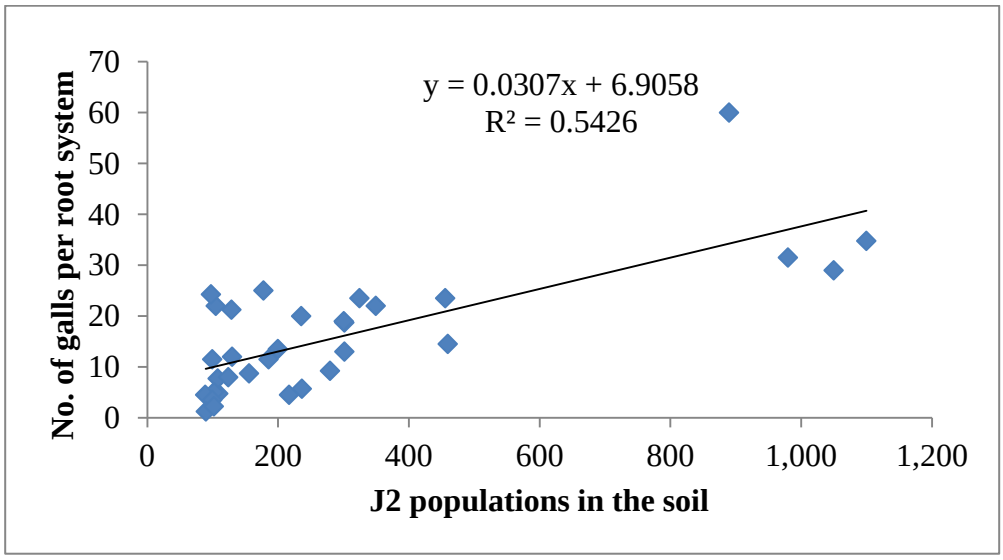


Figure 4.6: Relationship between J2 populations in the soil and the galls counts per root system of tomato plants in greenhouse (Expt. 1)

4.4.2 Effects of fungal bio-control agents on galling and egg mass indices on tomato plants

There were statistical differences (P≤0.05) in galling index of RKN on tomato between the treated and untreated plants (Table 4.5). The highest GI was noted in the untreated plants. No significant (P>0.05) difference was observed in GI among the BCAs. A lower galling index (GI) was recorded with Velum prime® treated plants and was significantly different (P≤0.05) from that recorded in all the BCAs and untreated control. The lowest GI was noted in Bionematon® treated plants (Table 4.5).

Trichoderma harzianum 2 treated plants had the lowest EMI which did not vary significantly (P>0.05) from all other BCAs. However, it varied significantly (P≤0.05) from that recorded in Velum prime® treated plants (Table 4.5).

Table 4.5: Mean gall and egg mass indices on tomato plants treated with different treatments in the green house (Expt. 1)

Treatment	**Galling index**	**Egg mass index**
Velum prime®	1.06±0.23 c	1.00±0.22 c
T. harzianum 1	2.00±0.22 b	2.00±0.27 b
Bionematon®	1.88±0.27 b	1.88±0.27 b
T. harzianum 2	1.88±0.33 b	1.81±0.31 b
P. lilacinum	2.06±0.31 b	2.13±0.27 b
T. harzianum 3	2.06±0.31 b	2.00±0.29 b
T. afroharzianum	2.00±0.30 b	2.19±0.33 b
Untreated control	2.88±0.95 a	3.00±0.18 a
L.S.D	**0.76**	**0.76**
P	**0.0003**	**0.0005**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

On further analysis, there was a positive correlation (r=0.70, P≤0.05) between galls count per root system and the galling index (Figure 4.7).

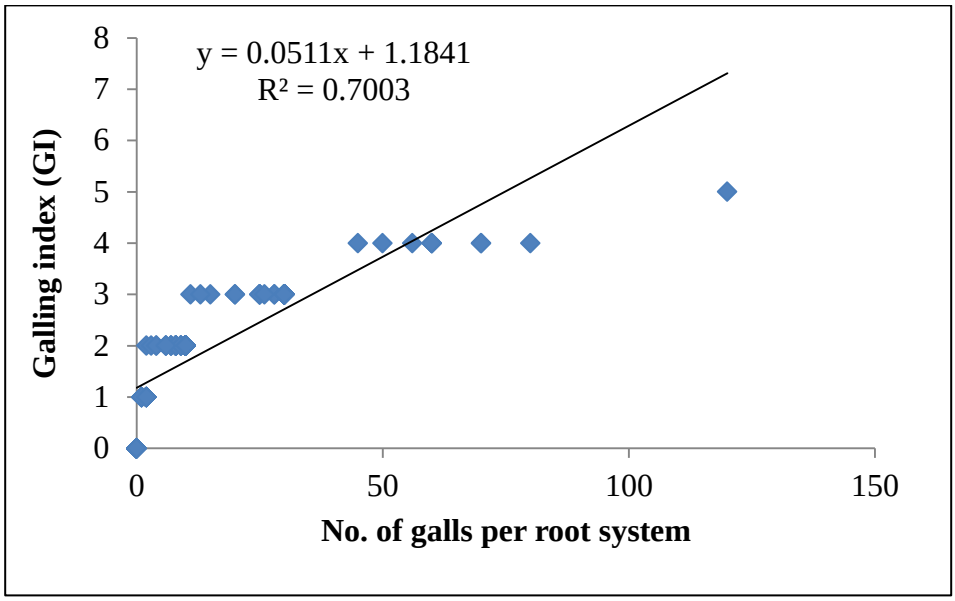


Figure 4.7: Relationship between the galls count per root system and galling index of Root-knot nematodes on tomato plants in the greenhouse (Expt. 1)

4.5 Effects of fungal bio-control agents on growth of tomato plants in the greenhouse (Expt. 2)

4.5.1 Effects of fungal bio-control agents on shoot height

The treated tomato plants were significantly ($P \leq 0.05$) taller than the untreated (Figure 4.8). The plants were noted to increase in height over time in all the treatments and the increase from 30-60 DAT was at a higher rate (80-97%) compared to 60-90 DAT (12-20%). At 30 DAT, no significant ($P > 0.05$) differences were noted in shoot heights among the bio-control agents (BCAs) including the commercial BCA (Bionematon®). Velum prime® treated plants had the tallest shoots throughout the test period and were significantly ($P \leq 0.05$) different the ones noted for plants treated with the BCAs and control at 60 DAT and 90 DAT.

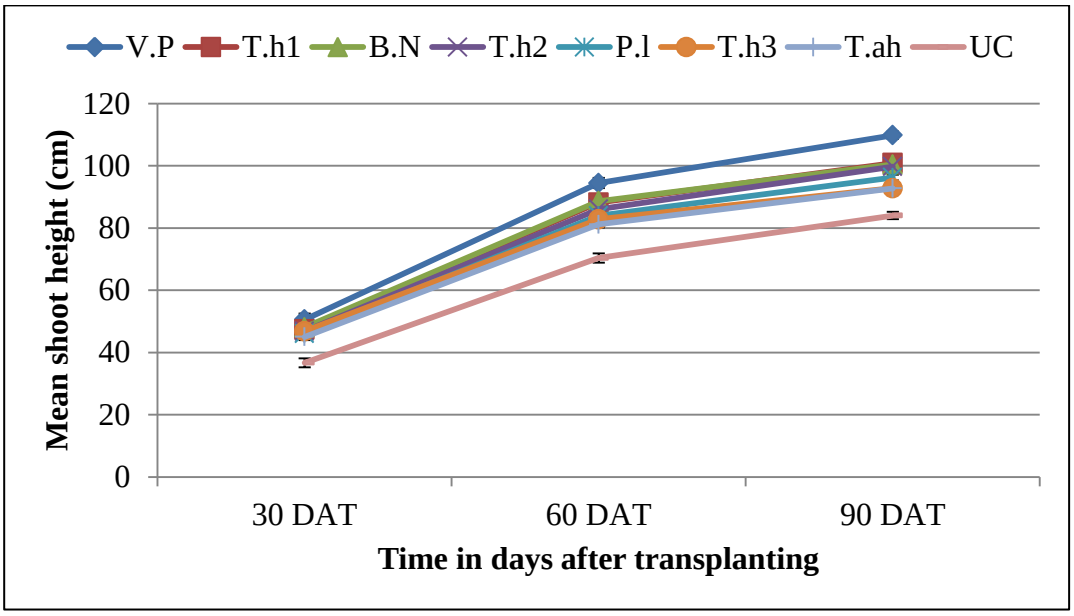


Figure 4.8: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the greenhouse (Expt. 2)

Data are mean of 4 replicates. V.P= Velum prime®, T.h1=*Trichoderma harzianum* 1, B. N= Bionematon®, T.h2= *T. harzianum* 2, T.h3= *T. harzianum* 3, T. ah=*T. afroharzianum*, UC=Untreated control.

Among the BCAs, *Trichoderma harzianum* 1 treated plants recorded the highest shoot heights over the test period and were significantly (P≤0.05) different from *P. lilacinum*, *T. harzianum* 3, and *T. afroharzianum* at 90 DAT (Figure 4.7). The shoot heights recorded in *T. afroharzianum* and *T. harzianum* 3 treated plants were significantly shorter (P≤0.05) than those recorded in the other BCAs over the test period. The shortest shoots were recorded in the untreated control. Overall, *T. harzianum* 1 performance was similar to that of Bionematon® and *T. harzianum* 2. *Trichoderma harzianum* 1 also was significantly (P≤0.05) better than *P. lilacinum*, *T. afroharzianum* and *T. harzianum* 3 treated plants in terms of shoot heights (Figure 4.8).

4.5.2 Effects of fungal bio-control agents on dry weight of shoot and roots (g), and root length (cm) of tomatoes

The plant dry weights of treated tomato plants were significantly (P≤0.05) higher than those recorded in untreated plants (Table 4.6). Velum prime® treated plants recorded significantly higher (P≤0.05) dry shoot and root weights than all the BCAs. Among the BCAs, *T. harzianum* 1 treated plants recorded higher dry shoot weights although this did not vary significantly (P>0.05) from the dry shoot weight recorded in *T. harzianum* 2 and *P. lilacinum* and Bionematon® treated plants.

In terms of dry root weights, *T. harzianum* 2 treated plants recorded higher dry root weights among the BCAs but was not significantly (P>0.05) different from Bionematon®, *P. lilacinum* and *T. harzianum* 1 treated plants. *Trichoderma harzianum* 3 treated plants recorded slightly lower root dry weights. The commercial *P. lilacinum* (Bionematon®) treated plants were not significantly (P>0.05) different from local isolate *P. lilacinum* in regards to dry shoots and roots weights. The untreated plants recorded the lowest dry weights in both roots and shoots (Table 4.6).

Table 4.6: Mean dry shoot and root weights (g) and root length (cm) of tomatoes under different treatments in green house (Expt. 2)

Treatments	**DSW**	**DRW**	**RL**
Velum prime®	8.69±0.15 a	5.80±0.27 a	19.23±0.29 a
T. harzianum 1	8.28±0.13 b	4.68±0.20 bcd	17.61±0.28 bc
Bionematon®	8.25±0.11 b	5.09±0.22 b	18.43±0.26 ab
T. harzianum 2	8.23±0.13 b	5.23±0.22 ab	18.30±0.18 b
P. lilacinum	7.98±0.11 bc	4.79±0.21 bc	17.32±0.33 cd
T. harzianum 3	7.74±0.13 cd	4.14±0.19 d	16.58±0.24 d
T. afroharzianum	7.58±0.12 d	4.48±0.20 cd	17.31±0.18 cd
Untreated control	6.33±0.22 e	3.45±0.18 e	13.12±0.48 e
L.S.D	**0.40**	**0.60**	**0.82**
P	**0.0001**	**0.0001**	**0.0001**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

DSW=Dry shoot weight, DRW=Dry root weight, RL=Root length.

The results also demonstrated significant ($P \leq 0.05$) differences in root length (cm) between the treated and untreated tomato plants (Table 4.6). The longest roots were recorded in Velum prime® treated plants. This was not significantly ($P > 0.05$) different from root lengths recorded in Bionematon® treated plants. Among the fungal isolates, *T. harzianum 2* recorded slightly longer roots followed by *T. harzianum* 1 and was significantly ($P \leq 0.05$) different from those recorded in *P. lilacinum*, *T. harzianum* 3 and *T. afroharzianum*. The shortest roots were noted in the untreated control (Table 4.6).

4.5.3 Effects of fungal bio-control agents on yield of tomatoes

The results of the study had notable differences in yield (Kg/plant) of tomatoes between the treated and untreated plants (Figure 4.9). The highest marketable yield was recorded with Velum prime® and was significantly ($P \leq 0.05$) higher than all the other treatments. Among the BCAs, *T. harzianum* 1 had higher yield which was not significantly ($P > 0.05$) different from *T. harzianum* 2, Bionematon® and *P. lilacinum*. *Trichoderma harzianum* 3 and *T. afroharzianum* had lower yield compared to the other BCAs but differed significantly ($P \leq 0.05$) from the untreated control (Figure 4.9).

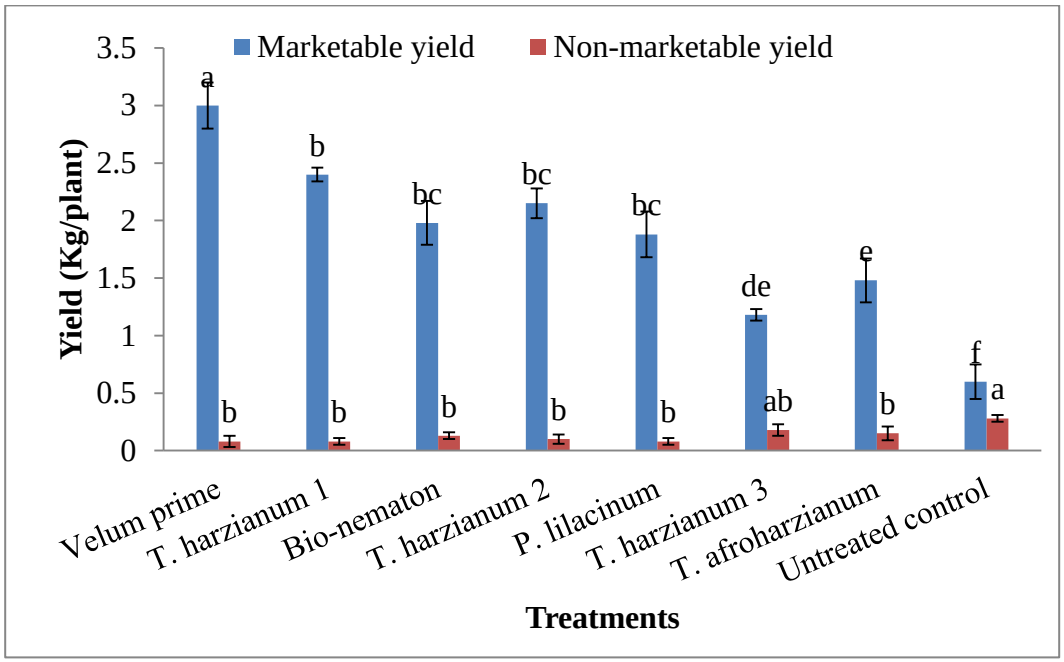


Figure 4.9: Mean yield (kg/plant) of tomatoes in the greenhouse (Expt. 2)

Data are mean ±SE of 4 replicates. Means followed by the different letter (s) are significantly different (P≤0.05) with LSD test.

Correlation analysis revealed a significant negative relationship (r=-0.77, P≤0.05) between the total yield of tomatoes and juvenile population at harvest (Figure 4.10).

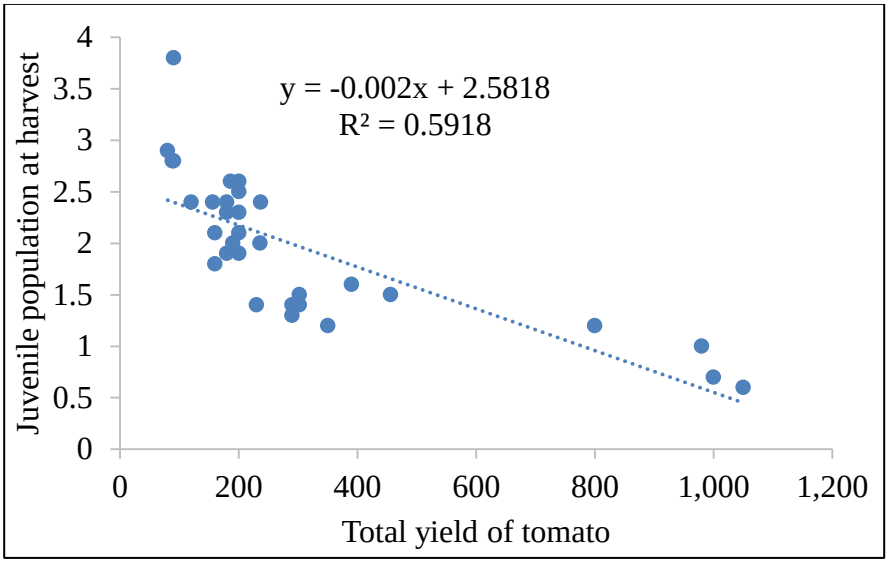


Figure 4.10: Relationship between the yield and juvenile population at harvest in greenhouse (Expt. 2)

4.6 Effects of fungal bio-control agents on root knot nematode (*Meloidogyne* spp.) disease components in the greenhouse (Expt. 2)

4.6.1 Effects of fungal bio-control agents on second stage Juveniles (J2) populations

The treatments significantly (P≤0.05) reduced the J2 populations in the soil over time while an increase was observed in the control (Table 4.7). At 30 DAI, Velum prime® treated plants recorded significantly (P≤0.05) lower J2 population than all the other treatments. On the other hand, *T. harzianum* 1 had a lower J2 population though not significantly different (P>0.05) from *T. harzianum* 2, *T. harzianum* 3 and *T. afroharzianum*.

Table 4.7: Mean J2 populations in the soils treated with different treatments at 30, 60 and 90 days after inoculation in the greenhouse (Expt. 2)

Treatments	**Mean J2/200cc of soil**		
	30 DAI	**60 DAI**	**90 DAI**
Velum prime®	290.75±33.68 d	229.75±18.75 d	87.00±2.38 d
T. harzianum 1	465.75±23.34 c	287.57±36.45 cd	171.50±7.67 cd
Bionematon®	575.00±36.63 b	329.75±24.06 cd	175.00±9.57 cd
T. harzianum 2	516.75±23.39 bc	322.75±46.02 cd	193.25±17.13 c
P. lilacinum	577.25±13.14 b	342.00±53.49 bcd	220.00±23.45 c
T. harzianum 3	516.25±37.09 bc	424.00±64.58 bc	311.00±13.30 b
T. afroharzianum	510.75±39.08 bc	489.50± 94.07 b	328.00±56.49 b
Untreated control	768.25±58.56 a	860.00± 53.39 a	957.50±54.52 a
L.S.D	**103.54**	**145.01**	**89.93**
P	**0.0001**	**0.0001**	**0.0001**

Data are means ± se. Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DAI: Days after inoculation

At 60 DAI, Velum prime® treated plants recorded a lower J2 population than all the other treatments but was not significantly (P≤0.05) different from that recorded in *T. harzianum* 1, Bionematon®, *T. harzianum* 2 and *P. lilacinum*. *Trichoderma afroharzianum* treated plants had higher J2 population than the other treated plants and did not differ significantly (P>0.05) from *T. harzianum* 3 and *P. lilacinum* (Table 4.7).

At 90 DAI, Velum prime® treated plants also had low J2 population followed by *T. harzianum* 1 and they did not differ significantly (P>0.05) from J2 populations recorded in Bionematon® treated plants. The final J2 populations recorded in *T. harzianum* 3 and *T. afroharzianum* did not significantly (P>0.05) differ but were significantly (P≤0.05) higher than those in the other treated pots. The J2 population in the untreated control were significantly (P≤0.05) higher than the treated plants (Table 4.7). The regression analysis revealed a remarkable positive correlation (r=0.48, P≤0.05) between J2 populations in the soil and the galls count per root system of tomato plants (Figure 4.11).

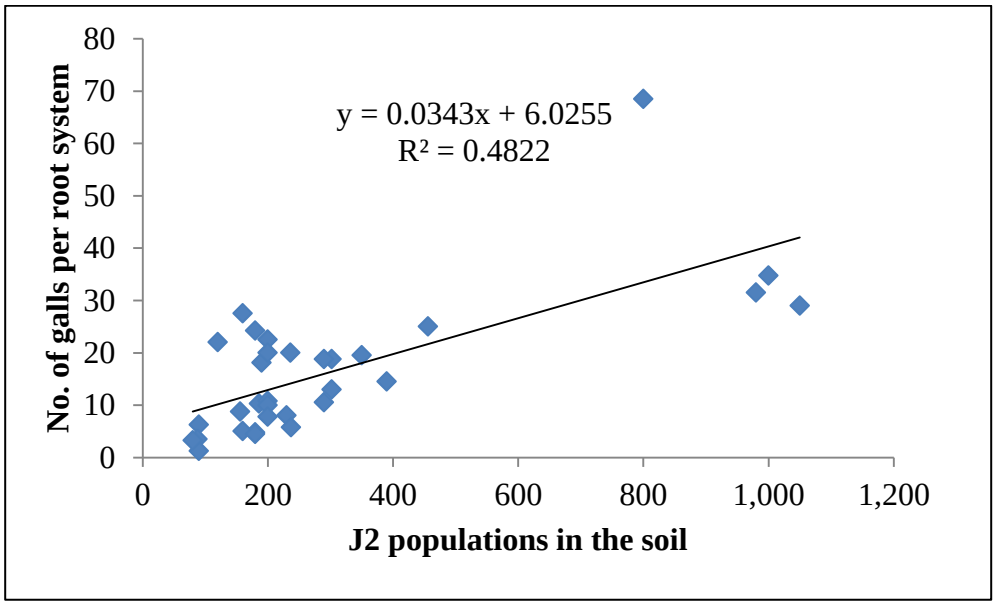


Figure 4.11: Relationship between J2 populations in the soil and the galls count per root system of tomato plants in greenhouse (Expt. 2)

4.6.2 Effects of fungal bio-control agents on galling and egg mass indices on tomato

All the treatments significantly (P≤0.05) lowered the disease severity of root-knot nematodes on tomato plants as depicted by the galling and egg mass indices (Table 4.8). The untreated plants recorded the highest GI. No significant (P>0.05) variations

were observed in GI amid the BCAs, however, a lower GI was noted in *T. harzianum* 2 treated plants. The lowest GI was recorded with Velum prime® treated plants and was significantly (P≤0.05) lower than that recorded in all the BCAs and untreated control (Table 4.8).

Table 4.8: Mean galling and egg mass indices on tomato plants treated with different treatments in the greenhouse (Expt. 2)

Treatment	**Galling index**	**Egg mass index**
Velum prime®	1.06±0.23 c	1.00±0.22 c
T. harzianum 1	2.00±0.22 b	2.00±0.27 b
Bionematon®	1.88±0.27 b	1.88±0.27 b
T. harzianum 2	1.88±0.33 b	1.81±0.31 b
P. lilacinum	1.94±0.23 b	1.94±0.23 b
T. harzianum 3	2.06±0.31 b	2.06±0.30 b
T. afroharzianum	2.00±0.30 b	2.19±0.33 b
Untreated control	3.19±0.23 a	3.06±0.19 a
L.S.D	**0.753**	**0.755**
P	**0.0001**	**0.0001**

Data are means ± se. Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

A similar trend to the GI was observed with EMI, where Velum prime® treated plants recorded significantly (P≤0.05) lower EMI compared to all the other treatments (Table 4.8). Among the BCAs, no significant (P>0.05) difference was observed in the EMI although *T. harzianum* 2 had a lower EMI. The highest disease severity in terms of EMI was noted in the untreated plants (Table 4.8). The regression analysis revealed the galls count and galling index of RKN on tomato plants were positively (r=0.81, P≤0.05) correlated (Figure 4.12).

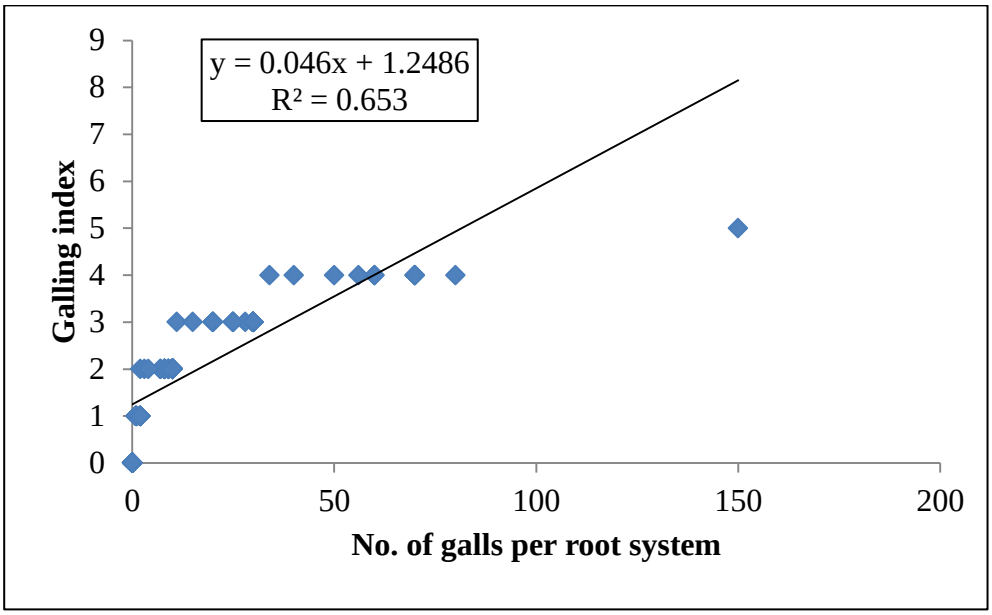


Figure 4.12: Relationship between the number of galls per root system and galling index of root-knot nematodes on tomato plants in the greenhouse (Expt. 2)

4.7 Effects of fungal bio-control agents on growth and yield of tomato plants in field season one

4.7.1 Effects of fungal bio-control agents on shoot height

The results revealed significant ($P \leq 0.05$) variations in shoot height of treated and untreated tomato plants (Figure 4.13). The plants were noted to increase in height over time in all the treatments and the increase from 30-60 DAT was at higher rate (53-77%) compared to 60-90 DAT (31-38%). Velum prime® treated plants recorded significantly ($P \leq 0.05$) taller shoots than all the other treatments during the test period. Among the bio-control agents (BCAs), *Trichoderma harzianum* 2 treated plants had taller shoots though not significantly ($P > 0.05$) distinct from the other BCAs at 30 DAT (Figure 4.13).

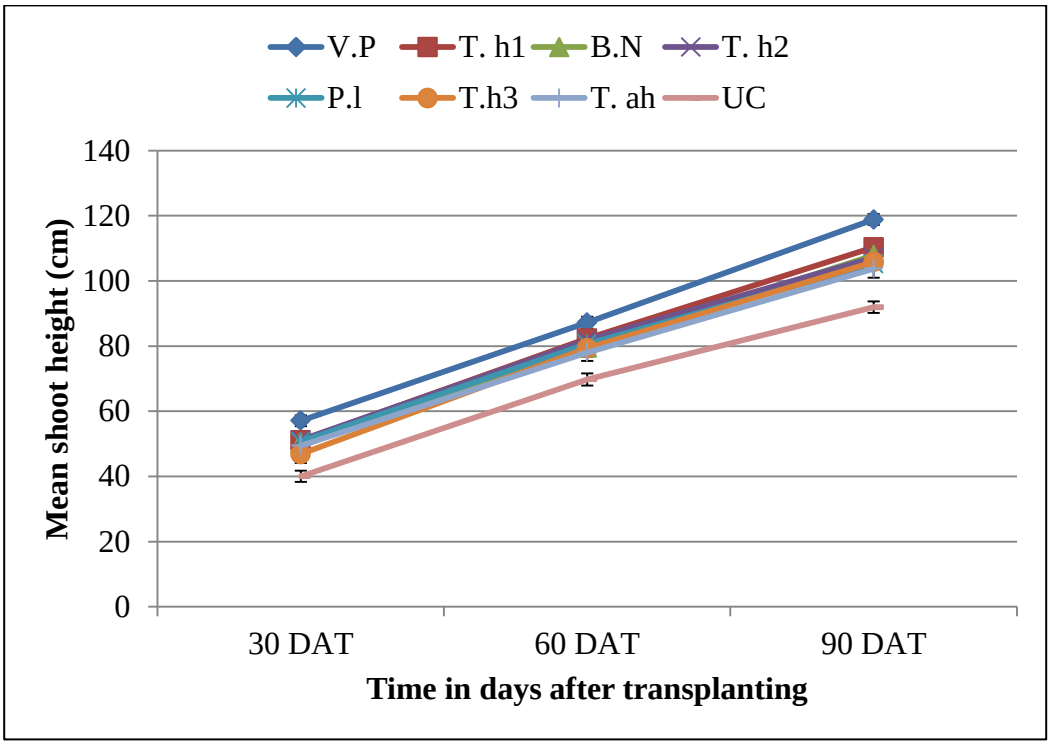


Figure 4.13: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the field (season one)

At 60 DAT, Velum prime® treated plants recorded higher shoot height than all the other treatments which was not significantly (P>0.05) distinct from that recorded in *T. harzianum* 1 and *T. harzianum* 2. There was no significant (P>0.05) difference in shoot height of plants treated with BCAs at 60 DAT. Velum prime® treated plants were the tallest throughout the season and significantly (P≤0.05) taller than all the other treatments at 90 DAT. *Trichoderma harzianum* 1 treated plants recorded higher shoots at 90 DAT among the BCAs including the commercial BCA (Bionematon®). It also had significantly (P≤0.05) taller shoots than *T. afroharzianum* treated plants. Although *T. afroharzianum* recorded a shorter shoot height it was statistically (P≤0.05) higher than the untreated control (Figure 4.13).

4.7.2 Effects of fungal bio-control agents on dry weights of shoot and roots (g) and root length (cm) of tomato plants

There were significant ($P \leq 0.05$) differences noted on dry plant weights between the treated and untreated tomato plants (Table 4.9). The highest dry shoot weights were recorded in Velum prime® treated plants which differed significantly ($P \leq 0.05$) from the other treatments. The commercial BCA (Bionematon®) treated plants had heavier shoots followed by *T. harzianum* 1 and did not vary significantly ($P > 0.05$) from *T. harzianum* 2, *T. harzianum* 3 and *T. afroharzianum*. However, these dry shoot weights were statistically ($P \leq 0.05$) distinct from *P. lilacinum* (Table 4.9).

Velum prime® treated tomato plants recorded heavier dry root weights though it was not significantly ($P > 0.05$) higher than that recorded in *T. harzianum* 2 treated plants (Table 4.9). Among the BCAs, *T. harzianum* 3 treated plants had the least dry root weights which differed significantly ($P \leq 0.05$) from *T. harzianum* 2 and was significantly higher than weights noted in untreated control. The commercial, *P. lilacinus* was not statistically different ($P > 0.05$) from *P. lilacinum* in terms of dry root weights, however recorded significantly higher dry shoot weights as shown in Table 4.9.

Table 4.9: Mean dry weights (g) of shoot and roots and root length (cm) of tomato plants under different treatments in the field (Season one)

Treatments	**DSW**	**DRW**	**RL**
Velum prime®	18.51±0.15 a	5.81±0.17 a	16.67±0.20 a
T. harzianum 1	17.71±0.28 b	5.00±0.20 bc	15.50±0.28 bc
Bionematon®	17.72±0.16 b	5.06±0.24 bc	15.83±0.29 b
T. harzianum 2	17.52±0.15 bc	5.29±0.23 ab	15.91±0.31 b
P. lilacinum	17.12±0.21 c	4.79±0.14 bc	15.08±0.23 c
T. harzianum 3	17.48±0.19 bc	4.66±0.16 c	15.02±0.19 c
T. afroharzianum	17.16±0.21 bc	4.90±0.21 bc	15.31±0.33 bc
Untreated control	10.41±0.21 d	3.73±0.13 d	13.22±0.18 d
L.S.D	**0.56**	**0.53**	**0.72**
P	**0.0001**	**0.0001**	**0.0001**

Data are means ± SE. Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DSW=Dry shoot weight, DRW=Dry root weight.

There was significant (P≤0.05) difference in root length between the treated and the untreated plants (Table 4.9). Velum prime® treated plants had longer roots and varied significantly (P≤0.05) from the other treatments. *Trichoderma harzianum* 2 attained longer roots among the BCAs followed by Bionematon® and were not significantly (P>0.05) distinct from *T. harzianum* 1 and *T. afroharzianum.* This, however, differed significantly (P≤0.05) from *P. lilacinum* and *T. harzianum* 3 (Table 4.9).

4.7.3 Effects of fungal bio-control agents on the yield of tomato

There was notable (P≤0.05) difference in the marketable yield (t/ha) of tomato plants between the treated and untreated plots (Figure 4.14). Velum prime® treated plants yielded significantly (P≤0.05) more fruit yield (t/ha) as compared to the other treatments.

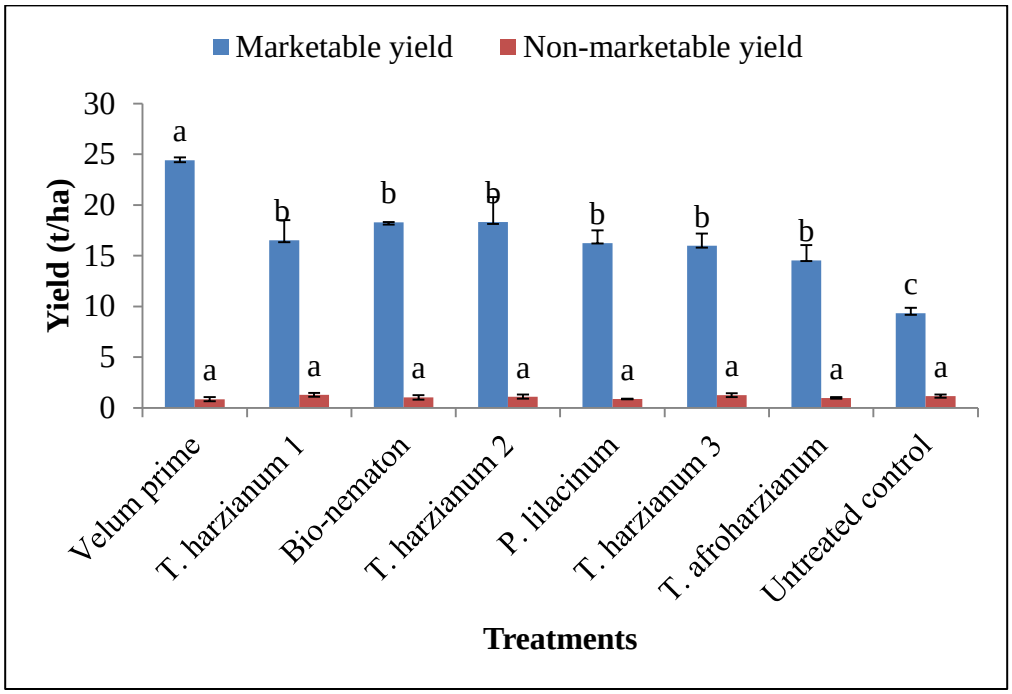


Figure 4.14: Mean tomato yield (t/ha) in field (Season one)

Data are mean of 4 replicates. Means followed by the same letter are not significantly different (P≤0.05) with LSD test.

No significant (P>0.05) difference was established in terms of marketable tomato yield among the BCAs, although they recorded significantly (P≤0.05) higher yield than the untreated control. No significant (P>0.05) variations were noted in non–marketable yield in all the treatments (Figure 4.14). On further analysis, a significant negative correlation (r=-0.58, P≤0.05) was revealed between the yield of tomato and juvenile population at harvest (Figure 4.15).

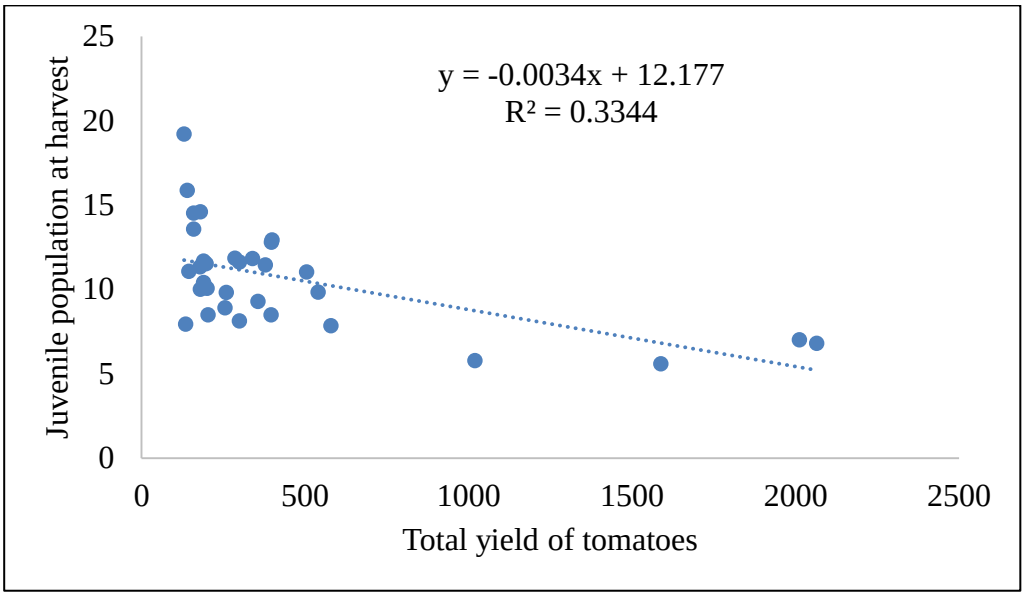


Figure 4.15: Relationship between yield of tomatoes and juvenile population at harvest in the field (season one)

4.8 Effects of fungal bio-control agents on root knot nematode (*Meloidogyne* spp.) on disease parameters in the field experiment (Season one)

4.8.1 Effects of fungal bio-control agents on J2 populations in the soil

The juvenile (J2) populations recorded before transplanting did not differ significantly (P>0.05) in all the treatment plots as shown in Table 4.10. The number J2 were noted to increase 30 days after transplanting (DAT) in all treatments. The J2 populations were effectively reduced in the treated plots as opposed to the untreated control where the J2 populations increased at 60 & 90 days after transplanting. A significantly (P≤0.05) lower J2 population at 60 & 90 DAT was noted in Velum prime® plots followed by Bionematon®. However, they were not significantly (P>0.05) different from J2 populations recorded in *T. harzianum* 1, 2 & 3 and *P. lilacinum*. *Trichoderma afroharzianum* on the other hand had a higher J2 population which was significantly (P≤0.05) lower than the untreated control (Table 4.10).

Table 4.10: Reproduction factor and Mean J2 populations in soils treated with different treatments at 30, 60 and 90 days after inoculation in the field (Season one)

Treatments	**Reproduction Factor**	**Mean J2/200cc of soil**			
		Initial populations	**30 DAI**	**60 DAI**	**90 DAI**
Velum prime®	0.56±0.19 c	385.00±129.39 a	475.50±151.39 b	277.75±43.13 c	143.75±6.25 c
T. harzianum 1	0.65±0.10 b	500.00±173.78 a	679.25±146.23 ab	504.75±121.90 bc	269.00±61.09 bc
Bionematon®	0.83±0.21 b	307.50±72.24 a	427.25±43.23 b	283.50±24.50 c	213.25±24.56 bc
T. harzianum 2	0.91±0.13 b	275.00±89.86 a	477.50±96.27 b	368.25±68.28 bc	229.25±56.12 bc
P. lilacinum	0.65±0.12 b	507.50±152.23 a	688.25±150.35 ab	490.00±100.25 bc	287.00±52.74 bc
T. harzianum 3	0.77±0.19 b	507.50±122.50 a	657.50±145.97 ab	514.00±83.64 bc	330.25±59.00 bc
T. afroharzianum	0.82±0.11 b	575.00±16.50 a	856.50±91.82 a	586.50±68.50 b	449.75±66.17 b
Untreated control	3.32±0.55 a	560.00±117.00 a	760.00±161.09 ab	1170.00±170.59 a	1671.50±241.88 a
L.S.D	**0.71**	**363.28**	**377.38**	**278.47**	**285.76**
P	**0.0001**	**0.5596**	**0.2537**	**0.0001**	**0.0001**

Data are means ± Se. Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DAI: Days after inoculation

There was a significant (P≤0.05) reduction in nematode reproduction factor in treated plots as opposed to the untreated plots (Table 4.10). A significantly (P≤0.05) lower RF was noted in Velum prime® treated plots compared to the BCAs treated plots and the control. Although *T. harzianum* 1 and *P. lilacinum* had a lower RF, no significant (P>0.05) difference was established in nematode RF among the BCAs (Table 4.10). The regression analysis revealed that the J2 populations in the soil and mean gall counts per root system of tomato plants were positively (r=0.71, P≤0.05) correlated (Figure 4:16).

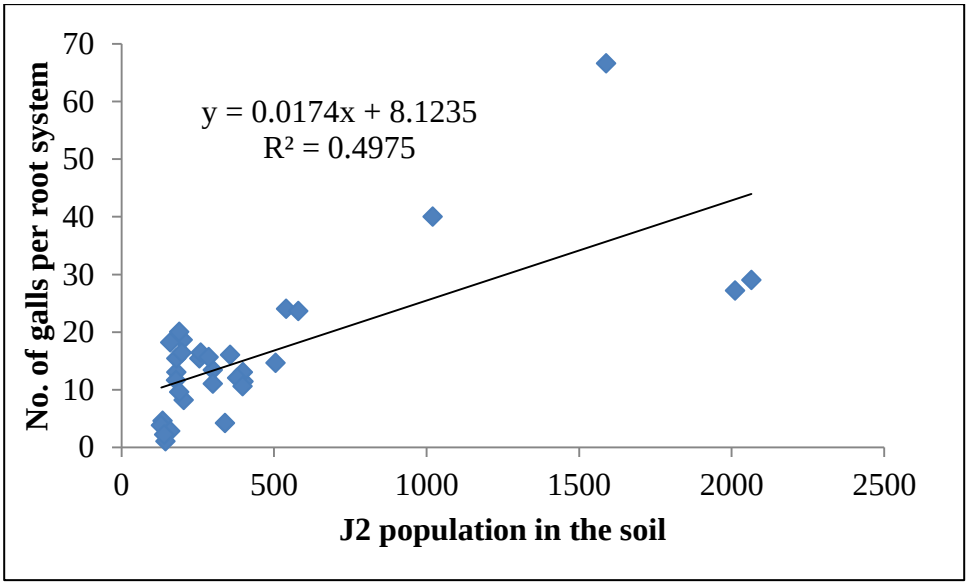


Figure 4.16: Relationship between the J2 populations in the soil and the number of galls per root system of tomato plants in the field (Season one)

4.8.2 Effects of fungal bio-control agents on galling and egg mass indices on tomato

There were significant (P≤0.05) variations established on egg mass and galling indices on tomato roots between the treated and untreated plants as shown in Table 4.11. The galling index (GI) of Velum prime® treated plants was significantly (P≤0.05) lower than all the other treatments. No significant (P>0.05) variations observed in the GI

recorded in all the BCAs treated plants, however, the GI was remarkably (P≤0.05) lower than that recorded in the untreated plants (Table 4.11).

Table 4.11: Galling and egg mass indices on tomato plants in soil under different treatments in the field (Season one)

Treatments	**Galling index**	**Egg mass index**
Velum prime®	0.95±0.20 c	0.85±0.20 c
T. harzianum 1	1.90±0.19 b	1.80±0.25 b
Bionematon®	2.00±0.23 b	1.95±0.22 b
T. harzianum 2	1.95±0.27 b	1.90±0.25 b
P. lilacinum	2.15±0.21 b	2.15±0.22 b
T. harzianum 3	2.05±0.26 b	1.85±0.24 b
T. afroharzianum	2.20±o.27 b	2.30±0.27 ab
Untreated control	3.15±0.20 a	2.90±0.16 a
L.S.D	**0.64**	**0.640**
P	**0.0001**	**0.0001**

Data are means ± SE. Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05.

The various treatments differed statistically (P≤0.05) in the Egg mass index (EMI) compared to the untreated plants and Velum prime® recording a lower egg mass index. Although a lower EMI was noted on *T. harzianum* 1 plants it did not vary significantly (P≤0.05) from that recorded on other plants treated with BCAs as shown in Table 4.11. It was further noted that the EMI recorded in *T. afroharzianum* was not significantly (P>0.05) lower than that recorded in the untreated plants (Table 4.11). Further analysis revealed a significant positive correlation (r=0.82, P≤0.05) between number of galls per root system and galling index of RKN on tomato plants (Figure 4.17).

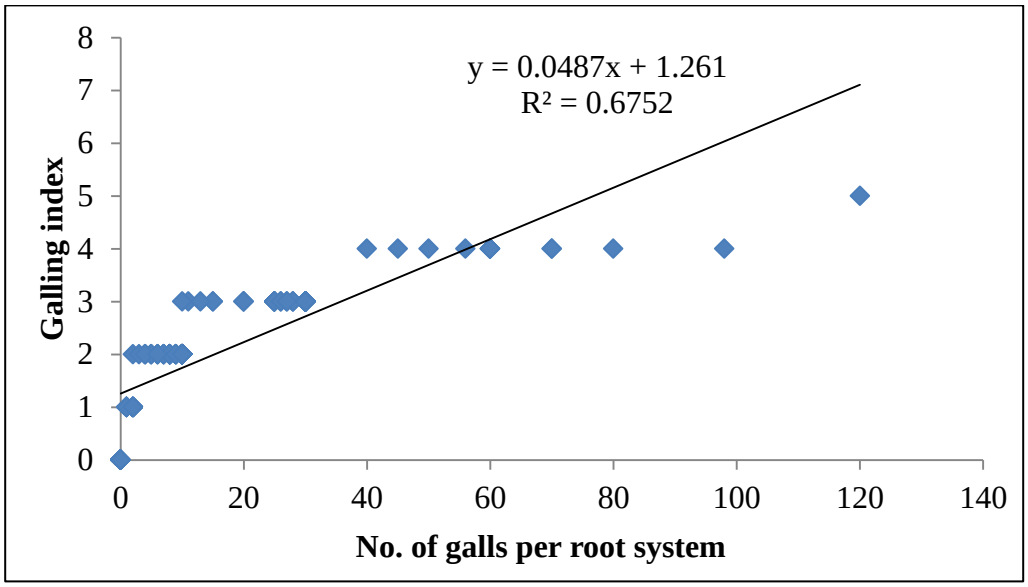


Figure 4.17: Relationship between the number of galls per root system and the galling index of root-knot nematodes on tomato plants in the field (Season one)

4.9 Effects of fungal bio-control agents on growth and yield of tomato plants in field (Season two)

4.9.1 Effects of fungal bio-control agents on shoot height

There were significant ($P \leq 0.05$) differences in shoot height between the treated and untreated tomato plants (Figure 4.18). Velum prime® treated plants recorded taller shoots throughout the test period and differed significantly ($P \leq 0.05$) in comparison to the other treatments at 90 DAT. The plants treated with the BCAs performed equally well as Velum prime® treated plants at 30 DAT.

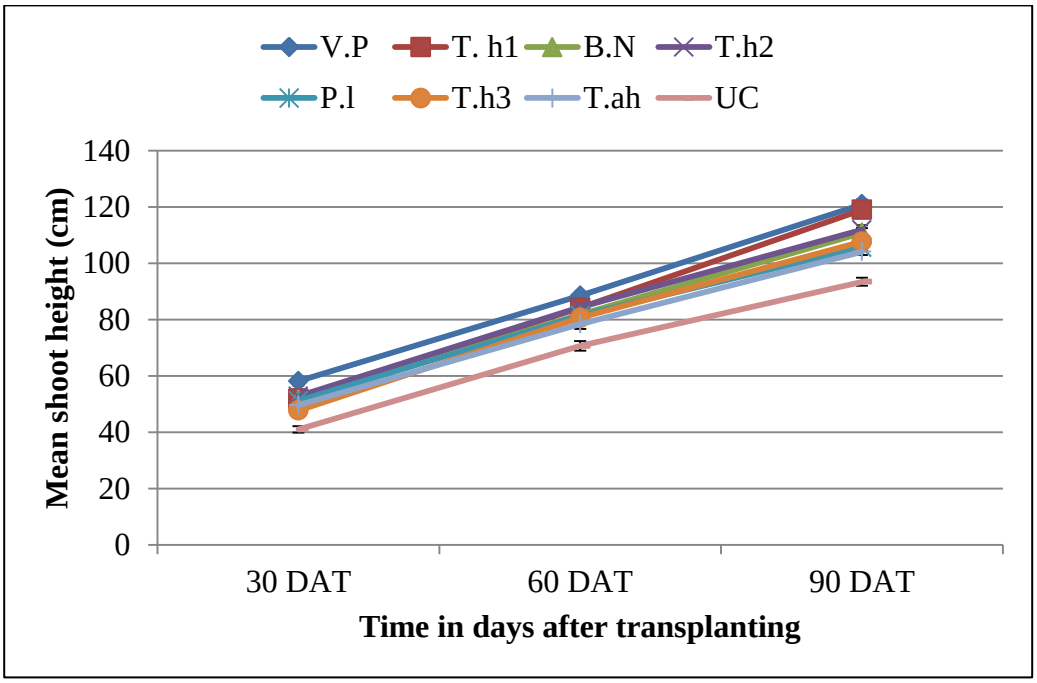


Figure 4.18: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the field (Season two)

Data are mean of 4 replicates. DAT=Days after transplanting, V. P= Velum prime®, T. h1=*Trichoderma harzianum* 1, B. N= Bionematon®, T. h2= *T. harzianum* 2, T. h3= *T. harzianum* 3, T. ah=*T. afroharzianum*, UC=Untreated control.

At 60 DAT, Velum prime® treated plants recorded higher shoot heights which were not significantly (P>0.05) different from that recorded with *T. harzianum* 1 and *T. harzianum* 2. *Trichoderma afroharzianum* recorded significantly (P=0.0001) shorter shoots than *T. harzianum* 1 and *T. harzianum* 2 (Figure 4.18).

Among the BCAs, *T. harzianum* 1 treated plants recorded taller shoots which were not significantly (P>0.05) different from those recoded with *T. harzianum* 2, *T. harzianum* 3 and Bionematon®. However, these differed significantly (P≤0.05) from *P. lilacinum* and *T. afroharzianum* at 90 DAT. The shoot heights recorded with *P. lilacinum* were significantly (P≤0.05) different from those recorded with Bionematon® (*Paecilomyces lilacinus*) treated plants at 90 DAT (Figure 4.18).

4.9.2 Effects of fungal bio-control agents on dry shoot and root weights (g) and root length (cm) of tomato plants

Overall there were significant ($P \leq 0.05$) differences in dry shoot and root weights between the treated and untreated tomato plants (Table 4.12). The heaviest dry shoot weights were recorded in Velum prime® treated plants which varied significantly ($P \leq 0.05$) from all the other treatments. Plants treated with *Trichoderma harzianum* 1 had heavier shoots than those treated with *P. lilacinum* and *T. afroharzianum*. However, they were not significantly ($P > 0.05$) distinct when compared to the plants treated with Bionematon®, *T. harzianum* 2 and *T. harzianum* 3. Among the BCAs, *P. lilacinum* treated plants recorded lower dry shoot weights which did not differ significantly ($P > 0.05$) from *T. afroharzianum* but was significantly ($P \leq 0.05$) different from the untreated plants (Table 4.12).

Velum prime® treated tomato plants recorded the heaviest dry root weights (Table 4.12). Among the BCAs, *T. harzianum* 2 treated plants recorded significantly ($P \leq 0.05$) heavier roots than *P. lilacinum* and *T. harzianum* 3. However, no variations were established in comparison to *T. harzianum* 1, Bionematon® and *T. afroharzianum.* The least dry weight of roots was recorded in the untreated plants as shown in Table 4.12.

Table 4.12: Mean dry shoot and root weights (g) and root length (cm) of tomatoes under different treatments in the field (Season two)

Treatments	**DSW**	**DRW**	**RL**
Velum prime®	18.71±0.78 a	5.96±0.15 a	16.89±0.20 a
T. harzianum 1	17.96±0.24 b	5.27±0.14 bc	15.71±0.25 bcd
Bionematon®	17.80±0.14 b	5.22±0.20 bc	15.96±0.25 bc
T. harzianum 2	17.65±0.15 bc	5.41±0.20 b	16.18±0.25 b
P. lilacinum	17.17±0.20 c	4.90±0.12 cd	15.21±0.21 d
T. harzianum 3	17.60±0.20 bc	4.73±0.16 d	15.09±0.19 d
T. afroharzianum	17.22±0.18 c	5.01±0.18 bcd	15.36±0.32 d
Untreated control	10.34±0.29 d	3.88±0.11 e	13.23±0.17 e
L.S.D	**0.53**	**0.45**	**0.66**
P	**0.0001**	**0.0001**	**0.0001**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DSW=Dry shoot weight, DRW=Dry root weight, RL=Root length.

The results of this study also revealed significant (P≤0.05) difference in root length between the treated and untreated plants (Table 4.12). Velum prime® treated plants recorded notably (P≤0.05) longer roots than all the other treatments. *Trichoderma harzianum* 2 recorded higher root lengths among the BCAs, but did not vary significantly (P>0.05) from plants treated with Bionematon® and *T. harzianum* 1. However, these were significantly (P≤0.05) different from *P. lilacinum*, *T. harzianum* 3 and *T. afroharzianum*. The untreated plants recorded significantly (P≤0.05) shorter roots than the other treatments (Table 4.12).

4.9.3 Effects of fungal bio-control agents on tomato yield

There was significant (P≤0.05) difference in the marketable yield (t/ha) of tomato plants between the treated and untreated plots (Figure 4.19). Velum prime® treated plants yielded significantly (P≤0.05) more fruits yield (t//ha) compared to the other treatments. Among the BCAs, *T. harzianum* 1 recorded more yield followed by *T.*

harzianum 2 which differed significantly (P≤0.05) from the yield in plants treated with *T. harzianum* 3. The lowest marketable yield was noted in the untreated plots.

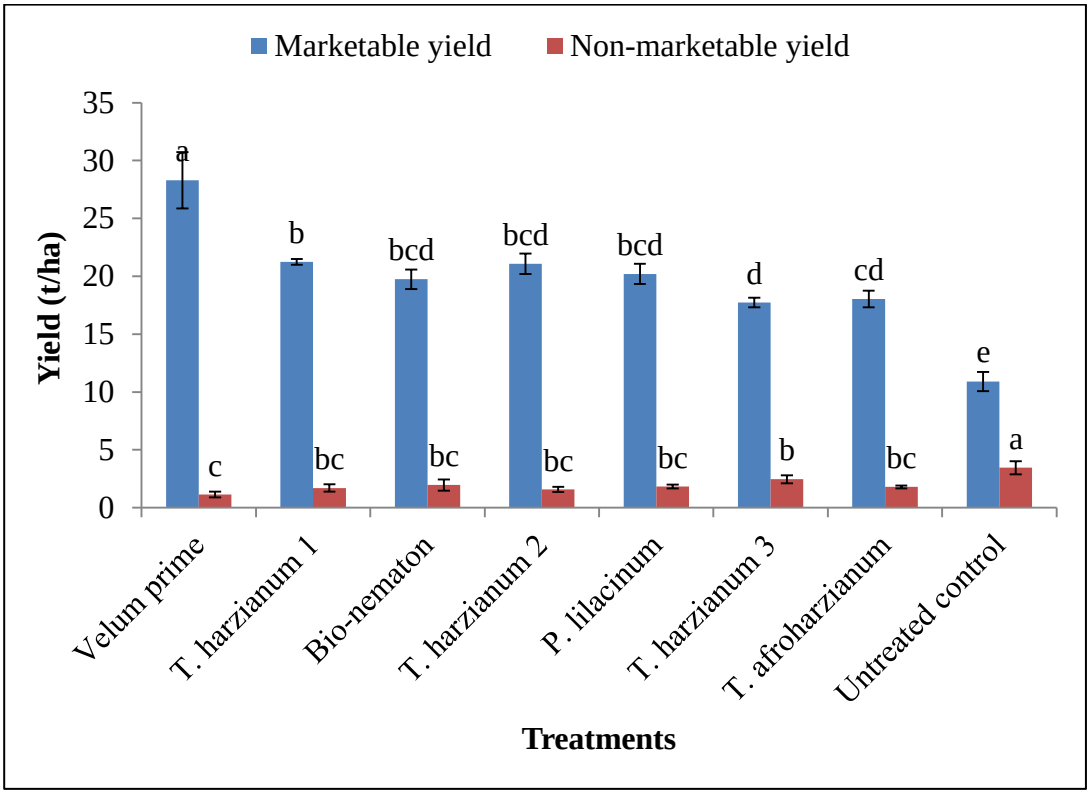


Figure 4.19: Mean tomato yield (t/ha) in field (Season two)

Data are mean of 4 replicates. Means followed by the same letter (s) are not significantly (P>0.05) different.

On the other hand, the untreated plots recorded significantly (P≤0.05) higher non- marketable yield. No significant (P>0.05) difference was noted in terms of non-marketable tomato yield among the BCAs, although they recorded a significantly (P≤0.05) higher non-marketable yield than the Velum prime® treated plants (Figure 4.19).

Correlation analysis revealed a significant negative relationship (r=-0.65, P≤0.05) between yield of tomatoes and juvenile population at harvest (Figure 4.20).

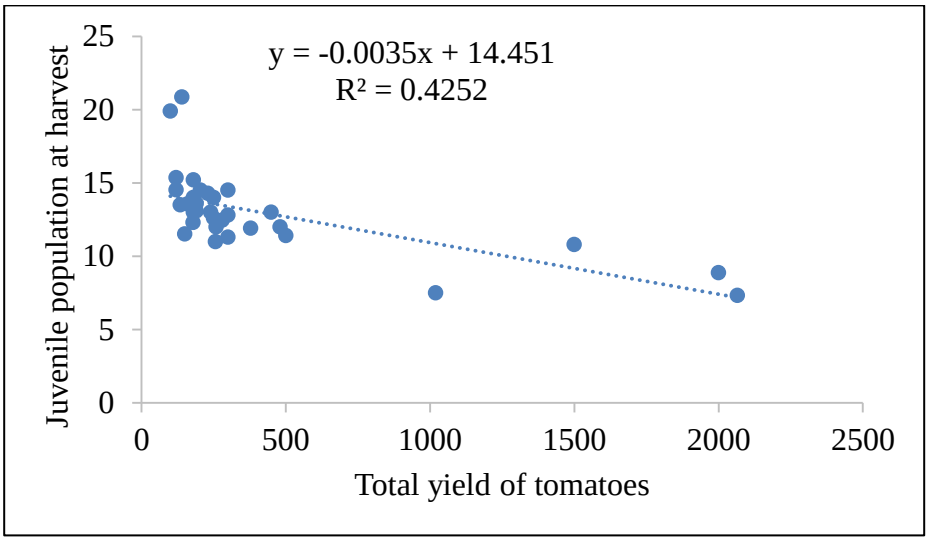


Figure 4.20: Relationship between yield of tomatoes and juvenile population at harvest in field (season two)

4.10 Effects of fungal bio-control agents on root knot nematode (*Meloidogyne* spp.) disease parameters during the field experiment season two.

4.10.1 Effects of fungal bio-control agents on J2 populations in the soil and reproduction factor

The Juvenile (J2) populations recorded at the start of the experiment did not differ significantly in all the treatment plots as indicated in Table 4.13. All the treatments were not found to suppress the J2 populations 30 days after transplanting (DAT). However, the J2 population in the treated plots was significantly ($P \leq 0.05$) suppressed at 60 and 90 DAT as compared to the untreated plots. At 60 DAT, the most suppressive treatment was Velum prime® but not distinct when compared to Bionematon®, *T. harzianum* 1 and 2 but it differed significantly ($P \leq 0.05$) from *T. harzianum* 3 and *T. afroharzianum*. Velum prime® also recorded a lower J2 population at 90 DAT which did not differ significantly ($P > 0.05$) from the BCAs apart from *T. afroharzianum*. The J2 populations were significantly ($P \leq 0.05$) higher in the untreated plots throughout the period and were noted to increase over time (Table 4.13).

Table 4.13: The reproduction factor and mean J2 populations in soils treated with different treatments 30, 60 and 90 days after inoculation in the field (Season two)

Treatments	**Reproduction factor (RF)**	**Mean J2/200cc of soil**			
		Initial populations	**30 DAI**	**60 DAI**	**90 DAI**
Velum prime®	0.52±0.18 b	300.00±74.27 a	428.00±109.78 c	237.75±22.36 c	120.00±8.17 c
T. harzianum 1	0.59±0.15 b	500.00±173.78 a	673.75±142.24 ab	399.50±79.27 bc	218.75±35.85 bc
Bionematon®	0.78±0.22 b	90.00±65.45 a	422.50±41.97 b	280.00±21.17 c	184.75±16.61 bc
T. harzianum 2	0.82±0.18 b	282.50±88.35 a	467.50±87.69 b	358.25±72.69 bc	192.50±19.74 bc
P. lilacinum	0.62±0.12 b	460.00±125.37 a	619.00±118.44 ab	472.50±85.92 bc	238.25±25.41 bc
T. harzianum 3	0.73±0.16 b	490.00±110.68 a	622.50±139.67 ab	477.50±87.69 b	314.00±55.94 bc
T. afroharzianum	0.78±0.12 b	555.00±96.31 a	836.25±82.96 a	567.50±62.37 b	407.25±43.52 b
Untreated control	3.34±0.55 a	545.00±113.62 a	847.50±146.14 a	1260.00±168.72 a	1646.25±243.94 a
L.S.D	**0.72**	**322.98**	**332.00**	**253.76**	**267.50**
P	**0.0001**	**0.3801**	**0.0696**	**0.0001**	**0.0001**

Means in the same column followed by the same letters are not significantly different according to Fisher's Least Significant Difference (LSD) test at P≤0.05. DAI=Days after inoculation.

There was significant (P≤0.05) difference noted on the effect of treatments on nematode reproduction factor compared to the untreated control (Table 4.13). A significantly (P≤0.05) lower RF was noted in the treated plots compared to the control. The isolated BCAs performed equally well as the Bionematon® and Velum prime® in reducing the nematode reproduction factor (Table 4.13). In addition, the J2 population in the soil and the galls count per root system were found to be positively (r=0.69, P≤0.05) correlated (Figure 4.21).

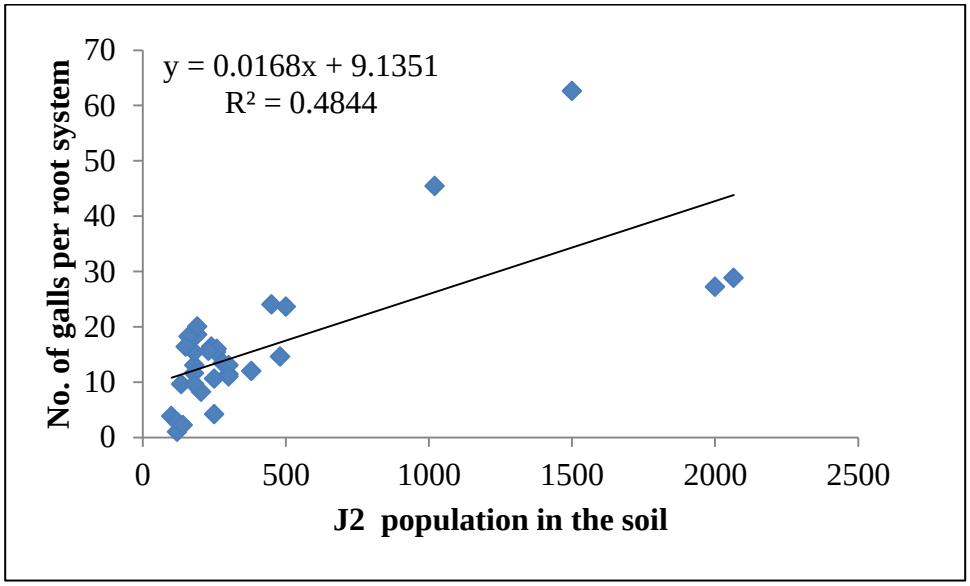


Figure 4.21: Relationship between J2 populations in the soil and number of galls per root system of tomato plants in the field (Season two)

4.10.2 Effects of fungal bio-control agents on galling and egg mass indices on tomato plants

There were highly significant (P≤0.05) variations established on the galling and egg mass indices on tomato roots between the treated and untreated plants as shown in Table 4.14. Velum prime® treated plants recorded significantly (P≤0.05) lower galling index (GI) from all the other treatments. No significant (P>0.05) variation was observed

in the GI recorded in all the BCAs treated plants, however the GI was significantly (P≤0.05) lower than that recorded in the untreated plants (Table 4.14).

Table 4.14: Galling and Egg mass indices on tomato plants in soil under different treatments in the field (Season two)

Treatments	Galling index	Egg mass index
Velum prime®	0.95±0.20 c	0.75±0.16 d
T. harzianum 1	1.90±0.19 b	1.65±0.22 c
Bionematon®	2.00±0.23 b	1.90±0.22 bc
T. harzianum 2	1.95±0.27 b	1.75±0.23 bc
P. lilacinum	2.15±0.21 b	2.00±0.19 bc
T. harzianum 3	2.10±0.27 b	1.75±0.23 bc
T. afroharzianum	2.55±0.31 b	2.35±0.28 b
Untreated control	3.45±0.18 a	3.00±0.18 a
L.S.D	**0.66**	**0.60**
P	**0.0001**	**0.0001**

Means followed by the same letter(s) within the same column are not significantly (P≤0.05) different according to Fisher's Least Significant Difference (LSD) test.

The tested treatments differed statistically (P≤0.05) in the egg mass index (EMI) recorded compared to the untreated plants (Table 4.14). Velum prime® recorded a significantly (P≤0.05) lower egg mass index than the other treatments. A lower EMI was noted on *T. harzianum* 1 treated plants followed by *T. harzianum* 2 and 3, and these did not vary significantly (P>0.05) from EMI recorded on Bionematon® and *P. lilacinum* treated plants (Table 4.14). It was further noted that *T. afroharzianum* recorded a higher EMI which differed significantly (P≤0.05) from *T. harzianum* 1 and was also significantly lower than untreated control (Table 4.14). It was further noted that the galls count per root system and galling index of RKN on tomato plants had a significant positive correlation (r=0.82, P≤0.05 (Figure 4. 22).

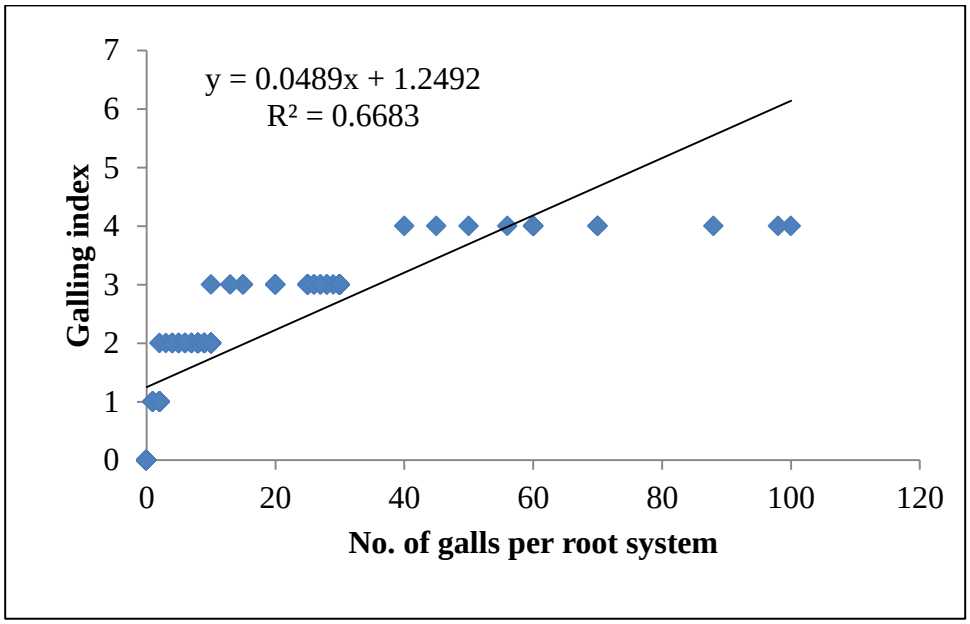


Figure 4.22: Relationship between the number of galls per root system and galling index of root-knot nematodes on tomato plants in the field (Season two)

CHAPTER 5: DISCUSSION

Fungi with antagonistic potential were isolated from tomato roots and RKN eggs sampled from Kirinyaga County. These isolates included *Trichoderma* spp., *Purpureocillium* spp., *Penicillium* spp., and *Fusarium* species. Similar fungal isolates have been reported from field grown tomatoes by other researchers in Kenya (Kariuki *et al*., 2012; Kibunja, 2015). Bogner *et al*. (2016) also isolated endophytic fungi from tomato roots collected from the Central and Coastal regions of Kenya including *Fusarium* spp., *Trichoderma* spp., *Alternaria* spp. and *Aspergillus* spp. Several other researchers have successfully isolated fungi including *Trichoderma* spp., *Fusarium* spp., *Acremonium* spp., *Aspergillus* spp., *Chemotium* spp., *Purpureocillium* spp. and *Pochonia* spp. from tomato roots and RKN eggs with bio control potential against root-knot nematodes (*Meloidogyne* spp.) *in vitro* (Affokpon *et al*., 2015; Lobna *et a*l., 2016; Silva *et al*., 2017b).

In this study the isolated fungi showed significant nematicidal activity through inhibition of egg hatching and mortality of the root-knot nematodes J2. Al Ajrami (2016) reported a reduction in egg hatch rate of *Meloidogyne javanica* after two and three days of exposure to 1500 and 3000 spore/ml of *P. lilacinus*. Kibunja (2015) also reported that indigenous isolates of *Trichoderma* spp. and *Aspergillus* spp. from coastal region of Kenya had significantly higher juvenile mortality rate when compared to the control. Singh and Mathur (2010) also isolated *Trichoderma* spp., *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp., and *Purpureocillium* spp. from egg masses of RKN obtained from tomato and egg plants and demonstrated their mortality of J2s *in vitro*. Silva *et al*. (2017a) also successfully isolated *Pochonia* and *Purpureocillium* species

from nematodes eggs and soils and found that they were able to inhibit J2 hatchability from *Meloidogyne enterolobii* eggs by direct contact under laboratory conditions.

The significant juvenile mortality could be due to various antagonism mechanisms exhibited by the fungal isolates. For example, *Trichoderma* spp., particularly *T. harzianum* is reported to act through antibiosis, mycoparasitism and secretion of cell wall degrading enzymes such as cellulase and chitinase to suppress *Meloidogyne* spp. (Harman *et al*., 2004). *Fusarium* spp. has been reported to control nematodes through egg hatching inhibition and J2 killing (Singh and Mathur, 2010).

The results of this study demonstrated that application of *Trichoderma harzianum* strains and *Purpureocillium lilacinum* isolates improved the growth of tomato through increased shoot height, length of roots and plant dry weight compared to the control under both green house and field conditions. Ahmed and Monjil (2019), observed *Purpureocillium lilacinum* increased plant height, number of leaves, root length and root dry weight of tomato plants in a pot experiment. Mukhtar (2018) demonstrated increased shoot height and length of tomato plants through application of *Trichoderma harzianum* and *T. viride*. However, he observed a decline in root weight of tomato depending on the dose which is in contrast with the current study findings. Khan *et al*. (2012) also recorded an improvement in the growth and yield of eggplant with bio-control agents; *Pochonia chlamydosporia*, *P. lilacinus* and *T. harzianum* through suppression of galls formation. In another study, inoculation of tomato plants with *T. harzianum* was shown to improve shoot length, root length, dry shoot mass and dry root mass (Nzanza *et al*., 2011). Lobna *et al*. (2016) also reported that soil inoculation with

T. harzianum enhanced the fresh shoot weight and plant length when compared with the control.

Our findings also agree with Ering and Simon (2018) who noted significant increase in growth and yield of tomato plants treated with *Trichoderma* isolates. Nzanza *et al*. (2012) also reported an increased tomato yield with application of *T. harzianum*.

The significant differences observed in tomato plant growth parameters following application of local fungal isolates (*Trichoderma* spp. and *Purpureocillium* spp.) could be due to one or more mechanisms of the isolates. Fungi may also produce toxic metabolites and antibiotics that inhibit nematodes and exclude other deleterious micro-organisms (Howell, 2003; Sikora *et al*. 2008). *Trichoderma* species can enhance growth through improved nutrient absorption, enhanced root growth, control of disease causing microorganisms or by getting rid of growth inhibitors from the soil (Shoresh *et al*., 2010). *Trichoderma* spp. has been proven to assist plants in tolerance to stress condition by enhanced root development (Mastouri *et al*., 2010). It participates in solubilising inorganic nutrients hence increased intake by the plants (Sharma and Pandey, 2009). *P. lilacinum* ability to suppress nematodes could have led to the increase in growth and yield of tomato. This hence indicates the yield increase was due to reduction in nematode multiplication.

The lower growth in untreated plants in the current study might be due to the stunting action of root-knot nematodes and unavailability of nutrients to the plants. *Meloidogyne* spp. was observed to readily infect tomato, retard the growth and lead to reduction in fresh and dry weights. Several authors have reported reduction in growth

due to infestation with root-knot nematodes (Mwangi *et al*., 2017). The galls on the roots could also disturb the important root functions like uptake and transport of water and nutrients hence reduced growth. Kankam and Adomako (2014) reported a decrease in plant height and weight due to inoculation of *Meloidogyne* J2 at 10 and 12 weeks after transplanting. The infective J2 invade the root and move to a site near the vascular tissue which disrupts intake of water and distribution of nutrients as (Hussey and Boerma 1989).

The results obtained from this study showed that the indigenous isolates of *Trichoderma* species and *Purpureocillium* species suppressed root-knot nematodes on tomatoes. This was explained by reduced *Meloidogyne* spp. population densities in the soil and reduction of nematode damage on tomato as supported by low gall index, egg mass index hence reduced nematode reproduction. The increase in juvenile population in the soil 30 days after transplanting could be explained by the fact that the fungal isolates take time to establish in the soil. The performance of the fungal isolates in suppressing RKN was comparable to the synthetic nematicides. This corroborates the findings by Ering and Simon (2018) who reported that *Trichoderma* isolates gave a level of nematode control comparable to Carbofuran synthetic nematicide. Kalele et al. (2010) reported that *P. lilacinus* strain significantly reduced the juveniles' population in both soil and roots of tomato plants when fungal inoculation was done at planting. The fungal strain also reduced galling index and *Meloidogyne* sp. multiplication rate. Mukhtar (2018) also observed that application of *T. harzianum* and *T. viride* led to a significant decline in the galls count, egg masses and reproduction of *M. incognita* depending on the dosage on tomato plants. *Paecilomyces lilacinus* was effective in

suppressing *M. javanica* by reduced root galling and egg mass production as indicated by gall index and egg mass index (Al Ajrami, 2016).

The reduction in nematode galls and egg masses in the current study may be attributed to the ability of the fungal isolates to easily inhabit the roots and hence reduce the feeding sites for nematodes. The lower number of galls could also mean the inability of most of the juveniles to enter the host roots. *Trichoderma* spp. action through mycoparasitism, antibiosis and competition that causes activation of plant defence by production of enzymes like glucanase have been studied (Howell, 2003; Vinale *et al*., 2008). Bio control agents (*Trichoderma* spp.) have been reported to cause reduction in nematode populations due to secretion of enzymes including chitinase, cellulose, protease and glucanase that take part in pathogen's cell wall degradation (Ritika and Utpal, 2014). *Trichoderma* spp. also has a specialized pressing organ (appressoria) that produces holes on the target organisms and thereby hyphae penetrate the tissues of the targeted organisms (Jena *et al*., 2017). The performance of *Purpureocillium lilacinum* may be attributed to the main mechanism as an egg parasite (Moosavi *et al*., 2010) that attack nematode eggs in the soil and release toxin lethal to the existing juveniles and those that may hatch later (Singh *et al*., 2013). *Purpureocillium lilacinum* can readily colonize root-knot nematodes eggs thus inhibit egg hatching and root penetration by *Meloidogyne* sp. juveniles (Cabanillas *et al*., 1988). Chitinase activity has also been associated with *P. lilacinum* and has been reported to destroy the cuticle and kill the nematodes (Morton *et al*., 2004).

The chemical nematicide Velum prime® was the most efficacious in enhancing the growth and also in controlling the root knot nematode in both greenhouse and field

conditions. Kumar et al. (2012) also reported that the chemical nematicide, Carbosulfan had the highest efficacy in increasing growth of plants, reducing the galls count as well as reproduction of the nematode compared to bio agents. Jena *et a*l. (2017) also reported that the chemical check, Carbofuran exhibited the highest improvement of growth and a decrease in galls count and nematode population in brinjal. He also noted the next best treatment to be *Trichoderma viride* after the chemical check. Although Velum prime® performed better, it was comparable to *Trichoderma harzianum* 1.

The current study also demonstrated differences in performance of the different *Trichoderma* isolates. Ering and Simon (2018) reported differences among *Trichoderma* isolates in reducing the juveniles' population and root galls/root system of tomato plants. The differences could also be attributed to the fact that the *Trichoderma* spp. have an extensive network of conidiophores that give rise to spores able to adhere to nematode at the various stages. These attachments and parasitic activity differs amid fungal species and strains (Sharon *et al*., 2007).

The higher performance of chemical nematicides (Velum prime®) could be attributed to its quick knockdown activity.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

According to the results of this study, indigenous fungal isolates from Kirinyaga County, Kenya including *Trichoderma* spp. and *Purpureocillium* spp. have the potential of controlling root-knot nematodes *in vitro* and *in vivo*. *Trichoderma harzianum* isolates were more effective than *Purpureocillium lilacinum* and *Trichoderma afroharzianum* in promoting growth and yield of tomato as well as root-knot nematodes control. The performance of the indigenous fungal isolates especially *T. harzianum* and *P. lilacinum* were comparable with the commercial bio control agents (*P. lilacinus*) contained in Bionematon® and in some instance *T. harzianum* performed better than the commercial BCA. The results also reveal that the synthetic nematicide (Velum prime®) was more effective than the bio control agents but was comparable to *T. harzianum* 1 isolates.

6.2 Recommendations

1. Based on the results of this study, the indigenous isolates *Trichoderma harzianum* and *Purpureocillium lilacinum* demonstrated effective antagonistic activity that can be further developed into a commercial product for use in integrated management of root-knot nematodes.
2. This study recommends further studies on the appropriate methods of application, timing and rates of application of the specific bio control agents.
3. Farmers should be sensitized on the biological control techniques in integrated nematode control.

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APPENDICES

Appendix 1: Approval of research proposal by the Graduate school

KENYATTA UNIVERSITY
GRADUATE SCHOOL

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

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School **DATE:** 31st July, 2018

TO: **Ms. Kariuki Ann Nyambura**
C/o Department of Agricultural Science & Technology

REF: A145/OL/CTY/32299/16

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 25th July, 2018, approved your Research Proposal for the M.Sc. Degree entitled, **"Efficacy of Indigenous Antagonistic Fungi Against Root-Knot Nematodes on Tomatoes in Kirinyaga 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 Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

HARRIET ISABOKE
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Agricultural Science & Technology Department

Supervisors:

1. Prof. Waceke Wanjohi
   C/o Department of Agricultural Science & Technology
   Kenyatta University

2. Dr. Maina Mwangi
   C/o Department of Agricultural Science & Technology Dept
   Kenyatta University

Appendix 2: NACOSTI permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
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Fax:+254-20-318245,318249
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Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/78706/28092**

Date: **12th February, 2019**

Anne Nyambura Kariuki
Kenyatta University
P.O Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Efficacy of indigenous antagonistic fungal against root knot nematodes on tomatoes in Kirinyaga County, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Kirinyaga County** for the period ending **12th February, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Kirinyaga County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kirinyaga County.

The County Director of Education
Kirinyaga County.