MOLECULAR CHARACTERIZATION AND NON-CHEMICAL MANAGEMENT OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) ON AFRICAN NIGHTSHADES IN SELECTED PARTS OF KENYA

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DECLARATION

I, Shem Bonuke Nchore, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or for any other award.

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Declaration by the supervisors

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

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DEDICATION

Dedicated to my father Mr. Joseph Nyakeramba Nchore Kerama and my mother Mrs. Jane Kemunto Nchore for their caring love, support and encouragement.

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ACRONYMS AND ABBREVIATIONS

AEZs	Agro-ecological zones
AFNS	African nightshades
ANOVA	Analysis of Variance
AVRDC	The World Vegetable Centre Regional Centre for Africa
a.s.l	Above sea level
dNTPs	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
HUB	Humboldt University of Berlin
IPGRI	International Plant Genetic Resources Institute
IPM	Integrated Pest Management
J2s	Second stage juveniles
KALRO	Kenya Agricultural and Livestock Research Organisation
MEGA	Molecular evolutionary genetic analysis
OAs	Organic amendments
PCR	Polymerase chain reaction
P_f	Final population
pH	Potential of Hydrogen ion concentration
P_i	Initial population
PPN	Plant parasitic nematodes
rDNA	Ribosomal deoxyribonucleic acid
Rf	Reproduction factor
RKN	Root-knot nematodes
rRNA	Ribosomal ribonucleic acid
SSU	Small subunit

ABSTRACT

Root-knot nematodes (RKN) (*Meloidogyne* spp.) cause up to 80 % yield losses in infected vegetables. A study was carried out to; assess the influence of farmers' knowledge and awareness on RKN damage on African nightshades (AFNS); assess the incidence and severity of RKN on AFNS; characterize the RKN species infecting AFNS; screen the AFNS for response to RKN and determine the efficacy of solarizing soils amended with selected organic materials against RKN. A root-knot nematode survey was carried out in selected farms in Lower midlands 1 (LM1), Upper midlands 1 (UM1), UM2, UM3 and UM4 located in Nandi, Bungoma, Kakamega and Uasin Gishu Counties during the April to July 2014 growing season. The survey revealed that 53.6 % of the AFNS farmers were not aware of RKN. Majority (66.7 %) of the farmers planted AFNS using organic manure while 33.3 % used inorganic fertilizers. Farmers controlled RKN through the use of pesticides, crop rotation, woodash and uprooting diseased crops. Two hundred and fifty soil and root samples were taken from depths of 20 cm from ten different points per farm to determine the disease incidence and severity. Incidence and severity of 94.13 % and 2.63 respectively was reported. Galling index ranging from 1.3 to 4.43 was reported. Molecular characterization identified *M. incognita*, *M. arenaria*, *M. hapla*, *M. javanica* and *M. lopezi* from the surveyed areas. The response of AFNS to RKN varied from resistant to susceptible. *Solanum eldoretiunum* and *S. scabrum* were resistant, while *S. sarrarachoides* was tolerant in the greenhouse and field conditions. *Solanum americanum* and *S. nigrum* line IP03 were resistant in the greenhouse, but were tolerant to RKN in both field experiments, while *S. nigrum* landrace from Kakamega and *S. opacum* were resistant in the greenhouse and field test at Kenyatta University but were tolerant to RKN at Chepterwai. Both *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 were susceptible in the field test at Chepterwai though they were tolerant in the field test at Kenyatta University. In addition, resistant and tolerant AFNS had lower RKN damage and reproduction compared to susceptible AFNS. Solarized soils amended with Cattle manure (Cm), *Tithonia diversifolia* (Td) and pymarc (Pm) reduced RKN population and damage significantly compared with non-solarized and non-amended controls. Solarization improved efficacy of Cm, Td and Pm against RKN reproduction and damage on *S. villosum*. Reproduction was lower on Cm, Pm and Td amended soils while galling index ranged from 0.7 to 2.2 in solarized soils compared to 1.4 – 5.0 in non-solarized soils. Sensitization of farmers on RKN damage and application of organic amendments to reduce disease incidence and severity is proposed. The dominant RKN identified threatens AFNS production in the surveyed regions. Farmers should grow tolerant AFNS on heavily infested soils to reduce RKN population and reproduction. The tolerant AFNS could also be used in breeding programs for the management of RKN. Solarizing soils amended with organic materials is an ideal integrated pest management strategy for combating RKN infecting AFNS.

CHAPTER 1: INTRODUCTION

1.1 Background of the study

African nightshades (AFNS) in the Solanaceae family and genus *Solanum* are widely distributed in the tropics (IPGRI, 2003). This family is made up of approximately 90 genera and up to 3000 species and is well distributed throughout the tropical and temperate regions of the world (Edmonds and Chweya, 1997). Five AFNS belonging to *Solanum nigrum* L., *S. scabrum* Miller, *Solanum physalifolium*, *S. americanum* Miller and *S. villosum* Miller are commonly grown in Kenya depending on the rainfall, availability of irrigation, soil texture and organic matter (Edmonds and Chweya, 1997; Schippers, 2002; Ministry of Agriculture, 2010).

African nightshades provide farmers in Western region of Kenya with a source of income for improved livelihood due to little capital investment required for their production (Olembo *et al*., 1995; Mertz *et al*., 2001). There is a high demand for AFNS vegetables in rural and urban areas in Kenya due to their nutritional value (Edmonds and Chweya, 1997; Schippers, 2002).

African nightshades contribute to food security and are good sources of micronutrients including iron, folate, iodine, selenium, zinc and vitamins A, B complex, C and E (Tindall, 1983; Schippers, 2002; Kamga *et al*., 2013; Abang *et al*., 2014). They are used in traditional and marriage ceremonies (IPGRI, 2003) besides playing significant role in treatment of ailments and healing stomach related ailments (Olembo *et al*., 1995; Manoko and Van der Weerden, 2004). In 2002, the Kenyan government acknowledged the potential of AFNS for the management of HIV/AIDS (Government of Kenya, 2002). Seeds of

AFNS are used for pigment extraction (Edmonds and Chweya, 1997). However, production of AFNS is faced with a myriad of constraints ranging from deteriorated soil fertility, poor seed quality, unpredictable weather patterns, pests and diseases (Abang *et al*., 2014). Among the pests infecting AFNS are plant parasitic nematodes (PPN) that includes the root-knot nematodes (RKN) in the genus *Meloidogyne* (Hussain *et al*., 2012). The RKN cause yield losses of more than US $ 78 billion per annum globally in economically important crops including vegetables (Siddiq, 2000; Agrios, 2005; Kaskavalci, 2007; Begum *et al*., 2014) than any single group of PPN. More than 90 species of *Meloidogyne* have been described, of which *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica* are the most important (Agrios, 2005; Onkendi *et al*., 2014).

Effective and eco-friendly management of RKN is a serious challenge for farmers (Nchore *et al*., 2012b). Crop rotation and nematicides are not always viable control options for these destructive pests. Chemical nematicides are unaffordable to small scale farmers despite their efficiency in the control of RKN (Haydock *et al*., 2006; Tariq, 2008). Besides, they are unavailable in the market due to their toxic nature and threat to the ecosystem (Kimenju *et al*., 2008; Sharma and Pandey, 2009; Wachira *et al*., 2009; Onkendi *et al*., 2014; Gine *et al*., 2016). Therefore, there is need for alternative eco-friendly methods of managing RKN damage in smallholder farming systems in Kenya.

Host resistance is one of the most effective, sustainable and economical component for RKN management (Anwar and McKenry, 2010; Hussain *et al*., 2014). Resistant varieties reduce nematode populations and reproduction and are also compatible to the environment (Starr and Roberts, 2004; Hussain *et al*.,

2014). Nchore *et al*. (2013) reported higher RKN population and reproduction on susceptible black nightshade varieties in Kenya.

Organic amendments have been recommended not only as alternative sources for improving vegetable yields (Abukutsa-Onyango, 2007), but also for management of RKN damage (Zarina, 1996; Waceke, 2001 & 2002; Viaene *et al*., 2006; Nchore *et al*., 2011; 2012a; Sumbul *et al*., 2015). Solarizing soils amended with organic matter including chicken manure, goat manure and cattle manure has been reported to improve the soil fauna besides reducing PPN infection (Viaene *et al*., 2006). These studies have not been reported in Kenya for the management of RKN on AFNS. Thus, one of the objectives of this study was to assess the efficacy of solarizing soils amended with selected organic materials for the management of RKN on AFNS.

1.2 Statement of the problem

Production of African nightshades is constrained by a myriad of challenges including its vulnerability to viral, fungal, bacterial and nematode diseases (Whitehead, 1997; Langer *et al*., 2015). Unlike the other pathogens, root-knot nematodes are more challenging to manage because they inhabit the soil and their symptoms are usually mistaken for water or mineral deficiency (Whitehead, 1997; Maleita, 2011).

Root-knot nematode (*Meloidogyne* spp.) is among the top five major plant pathogens and is ranked first in the world among the ten nematode genera considered as important pathogens (Mukhtar *et al*., 2013b) hence a serious threat to AFNS production. An estimated amount of US$ 500 million is spent on RKN control globally (Keren-Zur *et al*., 2000). *Meloidogyne* species cause

greatest yield losses ranging from 5 % to 60 % (Stirling *et al*., 1992). Root-knot nematode cause serious damage to AFNS impacting both the quantity and quality of marketable yields (Asif *et al*., 2015). Infected AFNS develop chlorosis, knotted roots, leaves drop and reduced growth causing severe yield losses and occassionary total crop failure.

Farmer's awareness of nematode pests and their management is essential in improving AFNS production in the country. However, the impact of farmers' knowledge, awareness and pest management strategies on RKN damage on AFNS has not been studied. This study therefore intended to assess how farmers' knowledge, awareness and pest management practices influence RKN damage on African nightshades.

Despite huge loses attributed to the RKN species on AFNS growing areas in Kenya, this nematode pest has not been fully characterized. Speedy and robust identification of RKN on AFNS is vital for proper decisions on their management to avert further yield losses and crop damage. The current molecular diagnostic tools for RKN are time consuming, labour intensive and require trained personnel (Onkendi *et al*., 2014). Therefore, there is need for a quick reliable diagnostic method. So far, there is no documented study carried out on RKN identification on AFNS in Kenya or elsewhere based on partial analysis of 18S rDNA gene that codes for SSU rRNA. This research endeavored to characterize RKN species infecting AFNS by targeting the 18S rDNA cistron.

The current RKN management strategies in Kenya rely on chemical nematicides, organic manure amendments and biological control. Additionally, management of RKN on AFNS based on host resistance and solarizing soils

amended with organic materials for management of RKN had not been evaluated. This study evaluated the response of available local and commercial AFNS against RKN in the greenhouse and field condition. In addition, the study also evaluated the effectiveness of solarizing soils amended with selected organic materials in reducing the RKN damage on African nightshades.

1.3 Justification

There are various RKN species occurring in vegetable growing regions in Kenya (Nchore *et al*., 2015). Root-knot nematode has a wide host range of over 5500 plant species including African nightshades (Trudgill and Blok, 2001; Nchore *et al*., 2013; Chitambo *et al*., 2016). Despite numerous studies reported on management of RKN in Kenya (Waceke and Waudo, 1993; Waceke, 2001 & 2002; Odour-Owino, 2002; Kariuki *et al*., 2010; Birithia *et al*., 2012; Nchore *et al*., 2011, 2012a, 2012b), there is no comprehensive study that has been carried out on the response of AFNS to RKN in the country or elsewhere and especially under the field and greenhouse conditions. Preliminary studies in Kisii and Trans-Mara Counties reported RKN reproduction factor ranging from 7.23 to 7.69 on *S. villosum* and *S. nigrum* landraces grown in that region (Nchore *et al*., 2012b).

Farmers in Kenya incorporate different organic materials into the soil during production of AFNS (Abuktsa-Onyango, 2007). However, farmers' lack of awareness of RKN damage also influences the damage of RKN on AFNS. Low input agriculture practiced by farmers leads to minimal use of nematicides and inorganic fertilizers hence influencing the damage of AFNS by the RKN.

Proper identification is key to effective management of RKN pests (Onkendi *et al*., 2014; Zeng *et al*., 2014). Using a single nematode isolate is a rapid and robust tool for diagnosis of RKN problems. Advancement in molecular studies and research in Kenya sheds light on the proper identification of RKN species reducing the cumbersome tasks of relying on morphological characterization techniques. Although studies by Muturi *et al*. (2012) reported *M. incognita*, *M. javanica* and *M. arenaria* pathogens infecting indigenous leafy vegetables in Kisii using DNA sequencing and isozyme phenotypes, there is no documented study carried out on RKN identification on AFNS in Kenya or elsewhere. Partial analysis of 18S rDNA gene that codes for SSU rRNA was therefore tested targeting the 18S rDNA cistron to identify the RKN species infecting AFNS. Additionally, plant resistance to phytoparasitic nematode infestation is one of the eco-friendly strategies for the management of RKN (Birithia *et al*., 2012; Nchore *et al*., 2013). Greenhouse studies by Nchore *et al*. (2012b; 2013) revealed that *S. nigrum* and *S. villosum* were susceptible and good hosts to RKN respectively. This study hence assessed the response of local and commercially available AFNS to RKN infection under greenhouse and field conditions.

Although studies have been carried out on efficacy of organic amendments in controlling RKN in AFNS (Nchore *et al*., 2011; 2012a; 2012b), none of them has evaluated their enhancement through solarization to improve their efficacy on the management of RKN in Kenya. Organic amendments have been used to manage plant parasitic nematodes (Waceke and Waudo, 1993; Bridge, 1996; Sumbul *et al*., 2015) with minimal negative effects on the environment (Hassan *et al*., 2010). Amending materials are abundant and readily available to the

farmers and are compatible and can be easily adopted into the farming system for management of RKN (Odour-Owino, 2002; Hassan *et al*., 2010). Combining soil solarization with organic amendments can increase the effectiveness of RKN control (Kaskavalci, 2007; Reddy, 2013). Solarizing soils amended with goat manure, cattle manure, chicken manure and brassicaceous materials releases toxic compounds and metabolites that reduce RKN populations (Sikora *et al*., 2005; Kaskavalci, 2007). However, this strategy has not been evaluated for the management of RKN in AFNS production systems in Kenya. Fields in Western Kenya are fallowed for six months from October to March during the dry season and thus these conditions are ideal for soil solarization. This study sought to assess the effect of solarizing soils amended with organic materials on RKN damage under the field conditions on *S. villosum* a susceptible AFNS that was identified during the screening experiment.

1.4 Hypotheses

i. Farmers' knowledge, awareness and pest management practices do not influence root-knot nematode damage on African nightshades.
ii. Root-knot nematodes do not infect African nightshades in selected Counties of Kenya.
iii. Root-knot nematode species infecting African nightshades in selected Counties in Kenya are not genetically diverse.
iv. The available local and commercial African nightshades are not resistant to root-knot nematodes.
v. Solarizing soils amended with selected organic materials does not improve efficacy against root-knot nematode damage on African nightshades.

1.5 Objectives

1.5.1 General objective

To characterize and develop sustainable non-chemical management strategies for root-knot nematode pests on African nightshades for improved yields and livelihood.

1.5.2 Specific objectives

i. To assess farmers' knowledge, awareness and pest management practices on root-knot nematodes damage on African nightshades.
ii. To assess the disease incidence and severity of root-knot nematode on African nightshade in selected Counties of Kenya.
iii. To characterize the root-knot nematode species infecting African nightshade.
iv. To screen the available local and commercial African nightshade for resistance against root-knot nematodes.
v. To determine the efficacy of solarizing soils amended with selected organic materials against root-knot nematodes on African nightshades.

1.6 Significance of the study

The study will create awareness about the best farming practices that improve African nightshade production and reduce root-knot nematode damage. The findings on RKN disease severity and incidence on AFNS could be useful for disease intervention leading to improved AFNS production. The findings on the dominant RKN species infecting AFNS could provide information

necessary for planning effective non-chemical management. Tolerant African nightshades to RKN identified will be used to improve vegetable production and further increase source of income, access to nutritive vegetables and food security in the country and reduce malnutrition in children. The study creates opportunities for generating new non-chemical technology that can easily be tapped and adopted into vegetable production system.

CHAPTER 2: LITERATURE REVIEW

2.1 Production and economic importance of African nightshades

African nightshades (AFNS) belonging to the Solanaceae family, occur in tropical and warm temperate regions from sea level to altitudes over 3500 m (Edmonds and Chweya, 1997). They tolerate some drought and do well on a wide range of soils, mostly on sandy to clay-loam which are deep and well drained with pH 5.5-7.0. African nightshades are grown and consumed in rural and urban set-ups in Kenya (Nchore *et al*., 2010). They are commonly grown in Nyanza, Western, Rift Valley and Coast provinces mainly for home consumption and urban markets (Ministry of Agriculture, 2010; Ondieki *et al*., 2011; Nchore *et al*., 2012a; Nchore *et al*., 2013). They are ranked third among the ten preferred leafy vegetables in Western Kenya (Abukutsa-Onyango, 2002).

African nightshade seeds are either broadcast or planted in rows at a depth of 0.25-5.0 cm (Tindall, 1983) at a spacing of at least 15 to 50 cm between plants (FAO, 1988) and 30-60 cm between rows. Germination occurs 10-15 days after sowing (Edmonds and Chweya, 1997). The optimum germination temperature is between 15 and 30 ^{o}C (Rogers and Ogg, 1981) and they are ready for harvesting three weeks after sowing (Abukutsa-Onyango, 2004). Farmers apply both organic and inorganic fertilizers during cultivation of AFNS not only to provide nutrients to the plants but also to improve soil texture and structure (Abuktsa-Onyango, 2007; Nchore *et al*., 2012a).

African nightshades are rich in health promoting compounds (IPGRI, 2003) and assist in combating micronutrient deficiencies and malnutrition. They have nutritional and medicinal attributes being rich in proteins, vitamins,

carbohydrates and other mineral elements (IPGRI, 2003; Kamga *et al*., 2013). They also contribute to food security and income generation among the smallholder farmers in Kenya and Africa (Ministry of Agriculture, 2010).

2.2 Farmers' knowledge of root-knot nematode and nematode pest management practices on the production of African nightshades

Majority of farmers in Sub-Saharan Africa lack knowledge of pests and diseases infecting vegetable crops in their farms (Abang *et al*., 2014; Auwal *et al*., 2015). Besides, very few farmers if any know RKN pests and diseases associated with them (Auwal *et al*., 2015). Studies by Muturi *et al*. (2012) and Nchore *et al*. (2012b) reported that AFNS farmers in Kisii and Trans-Mara were not aware of the RKN pest. Farmers were also reported to have contributed to the spread of the RKN through infected seedlings from their neighbours or market as well as through farm implements (Nchore *et al*., 2012b; Palomares-Ruis *et al*., 2014). Moreover, there were no effective mechanisms of controlling RKN that were reported by the farmers (Nchore *et al*., 2012b).

Farmers incorporate organic and inorganic fertilizers during cultivation of AFNS as source of nutrients to the plants as well as to improve soil texture and structure (Abukutsa-Onyango, 2007; Nchore *et al*., 2012a). Inorganic fertilizers confer some level of nematode control (Viaene *et al*., 2006). Application of some fertilizers may be toxic to nematodes or suppress their reproduction and damage through changes in host nutrition (Viaene *et al*., 2006).

Organic manures and inorganic fertilizers rich in K, N and P are important in nematode management. Interaction between K, N and P availability impacts nematode populations and damage (Viaene *et al*., 2006). Coyne *et al*. (2004)

reported that K is important in influencing the balance between N and P in the soil. Soils with higher levels of P and K tend to have higher RKN reproduction and damage (Coyne *et al*., 2004; Nchore *et al*., 2012a). Thus, application of inorganic fertilizers with higher levels of K and P to improve growth and production of AFNS increases RKN multiplication and damage. These farming practices have great influence on the soil structure and pH with organic amendments of animal origin conferring soils with alkaline and basic properties while inorganic fertilizers acidifying them. In addition, decomposition of organic materials release toxic chemicals like ammonia, phenolic compounds and carbon dioxide as well as high temperature necessary for nematode control (Stapleton, 1991; Sumbul *et al*., 2015; Giné *et al*., 2016).

Organic amendments improve the soil structure and texture hence bringing about control of diseases and RKN pest (Abang *et al*., 2014; Sumbul *et al*., 2015). Soil texture influences several significant properties to land use and management (Asif *et al*., 2015; Brown, 2015). Besides its influence on vertical and horizontal nematode movement within a field (Prot, 1979), soil texture also affects nematode survival, emergence and disease severity (Asif *et al*., 2015) which is directly related to water holding capacity and aeration.

Increase in human population exerts pressure on the available land constraining vegetable production. To achieve higher yields, farmers opt to control RKN with nematicides or intercrop AFNS with maize, beans or tomatoes (Per. Observation). Sharing of seedlings and farm implements is commonly practiced by farmers during vegetable production. Kimenju *et al*. (2009) reported that crop varieties, cropping system and poor farm management practices greatly influenced the spread of PPN within and between farms

leading to higher nematode infection. One of the objectives of this study was therefore to assess influence of farmers' knowledge, awareness and nematode management practices employed during production of AFNS on RKN damage.

2.3 Root-knot nematodes

2.3.1 Economic importance of root-knot nematodes

Root-knot nematodes (RKN) in the genus *Meloidogyne* belong to a relatively small but important polyphagous group of highly adapted obligate plant pathogens (Abu-Gharbieh *et al*., 2005; Karssen *et al*., 2013). They are distributed worldwide and parasitize more than 5500 plant species including almost all cultivated crops (Abad *et al*., 2003; Okeniyi *et al*., 2009).

Root-knot nematodes grow and reproduce within the roots of their hosts and induce small to large knots (Karssen *et al*., 2013). Root-knot nematodes have marked sexual dimorphism with the males being vermiform and active, while the females are pyriform or saccate and sedentary (Tariq, 2008). Mature females measure between 0.5–1.5 mm in length and 0.33–0.7 mm in width (Taylor and Sasser, 1978; Beije *et al*., 1984). These nematodes inflict great losses to various vegetable crops and fruit trees (Abu-Gharbieh *et al*., 2005; Tariq, 2008) causing yellowing and stunted growth, root deformation and damage. When the roots are attacked, knots are formed interfering with uptake of water and mineral salts hence affecting foliar growth on many host plants (Anwar and McKenry, 2010).

More than 90 *Meloidogyne* species have been described (Subbotin *et al*., 2013; Onkendi *et al*., 2014) of which 22 species have been reported in Africa and five of them have been recognized as major and widely distributed in

Kenya (Beije *et al*., 1984; Onkendi *et al*., 2014; Chitambo *et al*., 2016). These are *Meloidogyne incognita* (Kofoid and white) Chitwood, *M. javanica* Treub, *M. arenaria* Neal, *M. enterolobii* and *M. hapla* Chitwood (Sasser, 1980). These species are responsible for at least 90 % of all damage caused on plants by RKN worldwide (Ateeq-ur-Rehman, 2009).

Root-knot nematodes are pests of major economic importance associated with vegetable crops globally (Hassan *et al*., 2010; Onkendi *et al*., 2014) causing severe damage and substantial yield losses ranging between 5 and 12 % globally (Sikora and Fernandez, 2005). In some instances total crop failure may result (Hassan *et al*., 2010). Siddiqi (2000) reported reduction of vegetable yields by RKN ranging from 10 to 80 % resulting in large economic losses in vegetable crops.

Root-knot nematodes live within plant roots or inhabit the rhizosphere soil around plant roots and root hairs (Agrios, 2005). So far, 13 nematodes including RKN attacking black nightshades (*Solanum nigrum*), broad leaved nightshade (*S. scabrum*) and American nightshade (*S. americanum*) have been reported (Rogers and Ogg, 1981; Lamodia, 1996; Zancada *et al*., 1998; Kutywayo and Been, 2006; Castillo *et al*., 2008; Anwar *et al*., 2009; Dorman and Nelson, 2012; Gharabadiyan *et al*., 2012; Muturi *et al*., 2012; Nchore *et al*., 2012b; Ardakani and Mirinejad, 2013; Chitambo *et al*., 2016). In addition, RKN are spread by transplanting infested seedlings, soil, farm implements, via irrigation water and sticking onto farm workers' feet (Beije *et al*., 1984).

Preliminary findings revealed that RKN is a pest of economic importance in black nightshade (*S. nigrum*) causing substantial damage to the roots (Nchore *et al*., 2012a; 2013). The study revealed that RKN reproduces on black nightshade

varieties reported in Kisii and Trans-Mara Counties of Kenya with reproductive factor >7 (Nchore *et al*., 2012a). Yield loses due to RKN range from 5 to 60 % (Stirling *et al*., 1992; Rivera and Aballay, 2008) in vegetable crops.

Root-knot nematode reproduction and infestation is influenced by prevailing environmental and edaphic factors (Chitwood and Perry, 2009). This factor together with the tendency of farmers to practice monocultivation of AFNS increases the rate of RKN infestation (Mbogoh *et al*., 2013). Root-knot nematodes prevalence on Solanaceae plants exceeds 50 % in Western region (Jogallo, 1984; Arim *et al*., 2006; Nchore *et al*., 2012a). There is need therefore to assess RKN damage on the various AFNS from other areas for documentation and intervention. Similarly, there is need to identify the species of RKN prevailing in the study area to assist in designing a sustainable RKN management strategy.

2.3.2 Life cycle and behaviour of root-knot nematodes

Root-knot nematodes have a short life cycle of six to eight weeks (Pakeerathan *et al*., 2009). This enables them to have higher reproduction and survival rates resulting to severe crop losses in warmer tropical countries (Ateeq-ur-Rehman, 2009). Root-knot nematodes may reproduce by obligatory mitotic parthenogenesis (*Meloidogyne incognita*, *M. javanica* and *M. arenaria*), amphimixis (*M. kikuyensis*, *M. pini* and *M. spartinae*) or by facultative meiotic parthenogenesis (*M. hapla*, *M. chitwoodi* and *M. fallax*) and have several generations on one crop (Tariq, 2008; Chitwood and Perry, 2009).

The female lay between 1000 and 3000 eggs enclosed in gelatinous egg sacs (Maggenti and Allen, 1960). The eggs are deposited on the surface of galled

roots or sometimes they occur within the galls (Karssen *et al*., 2013). Following embryogenesis, the first moult occurs within the egg giving rise to the infective second-stage juveniles (J2s) (Maleita, 2011; Karssen *et al*., 2013). Hatching of *Meloidogyne* eggs is temperature driven and occurs devoid of stimulus from plant roots; however, root diffusates sometimes stimulate hatching (Karssen *et al*., 2013). The egg shell becomes flexible immediately before hatching and enzymes are thought to be involved in altering egg shell structure. The J2 and the males are the stages of *Meloidogyne* that can be found freely in the soil (Karssen *et al*., 2013). Moreover, the J2 can survive in the soil in a quiescent state for an extended period of time consuming the food reserves in their intestines (Maleita, 2011; Karssen *et al*., 2013).

Many organic and inorganic compounds excreted by roots form gradients from the root surface into the soil and may influence the nematodes. Carbon IV Oxide is the most important factor for attracting RKN (Karssen *et al*., 2013). When RKN come into contact with plant roots, they often penetrate immediately (Maleita, 2011). Penetration occurs directly behind the root cap (Karssen *et al*., 2013). The J2s penetrate the rigid root cell walls by a combination of physical damage via thrusting of the stylet and breakdown by cellulytic and pectolytic enzymes (Maleita, 2011; Karssen *et al*., 2013). This penetration process occurs between 10 ^{o}C and 35 ^{o}C with the optimum being at about 27 ^{o}C depending on the species (Beije *et al*., 1984). The enzymes dissolve the middle lamella interrupting the cell division (Maleita, 2011).

Following penetration, the root tip may enlarge and root growth often stops for a short period. The J2s migrate intercellularly to the cortex in the region of cell differentiation causing the cells to separate along the middle lamella. The

J2s migrate towards the root tip and turn around when they arrive in the apical meristematic region (Maleita, 2011; Palomares-Ruis *et al*., 2014). They then move back up in the vascular cylinder towards the zone of differentiation, become sessile in the cortical tissue in the zone of differentiation.

The head of the J2 is embedded in the periphery of the vascular tissue and the rest of the body is in the cortex parallel with the longitudinal axis of the root. The J2s increases in size and undergoes the second and third moult forming the non-feeding J3 and J4 respectively (Maleita, 2011). The J4 develops into either female or male. The male leaves the root and becomes free living in the soil while the female becomes sedentary and secretes growth regulators that induce the host plant to produce ethylene and auxin leading to giant nurse cells around its head (Maleita, 2011). Formation of giant cells interferes with vascular vessels in infected crops causing water deficiency, loss in vigour, chlorosis, stunted growth, reduced yields and plant death during hot dry weather (Sasser and Carter, 1985; Agrios, 2005). Mature female lay eggs and the cycle is repeated again. No eggs are laid below 14.2 ^{o}C or above 31.7 ^{o}C and a new generation can arise within 25 days although under less favourable conditions, the time may be prolonged for 30 to 40 days (Beije *et al*., 1984).

A short life cycle, existence of different species, ability to inhabit and attack underground parts of plants and high reproductive rates makes control and management of RKN difficult (Stirling, 1991; Sikora and Fernandez, 2005). The RKN are the most destructive and difficult pest to control in tropical and subtropical countries (Keren-Zur *et al*., 2000; Simpson and Starr, 2001).

2.3.3 Diversity and host range of root-knot nematodes

Species of RKN show a wide diversity in their life cycle. They can be divided into two distinct groups; thermophils and cryophils, based on their temperature requirements to survive lipid-phase transition at 10 ^{o}C (Moens *et al*., 2009). *Meloidogyne chitwood*, *M*. *hapla* and *M*. *naasi* are cryophils that survive at soil temperatures below 10 ^{o}C, while *M*. *javanica*, *M*. *arenaria* and *M*. *exigua* are thermophils that do not survive at lower temperatures.

Root-knot nematodes are members of the genus *Meloidogyne* (Moens *et al*., 2009). *Meloidogyne incognita*, *M*. *javanica*, *M*. *arenaria* and *M*. *hapla* are the most frequent root-knot nematodes present in almost all countries (Ornat and Sorribas, 2008; Moens *et al*., 2009). *Meloidogyne incognita*, *M*. *javanica* and *M*. *arenaria* are commonly found in tropical regions, while *M*. *hapla* is common in subtropical and temperate regions.

Root-knot nematodes show various degrees of specialization with respect to their host preference (Moens *et al*., 2009) with higher preference on cultivated crops than weeds (Mandulu and Trudgill, 1993). Root-knot nematodes are an important polyphagous group of highly adapted obligate pathogens attacking over 5500 plant species including vegetable crops (Trudgill and Blok, 2001). They reproduce and feed on modified living plant cells on a wide range of crops ranging from higher plants and cultivated crops to volunteer plants and weeds (Moens *et al*., 2009). Among the families of plants infected by *Meloidogyne* species includes Amaranthaceae (*Amaranthus* species), Solanaceae (*Solanum nigrum* L.), Gramineae, Cyperaceae, Euphobiaceae, Geraniaceae, Labiatae, Leguminosae, Malvaceae, Oxalidaceae, Polygonanaceae, Portulaccaceae, Primulaceae, Rosaceae and Urticaceae (Ornat and Sorribas, 2008). Most

amphimictic species have host ranges confined to a single subclass of plants on either woody or perennial herbaceous hosts. The automictic species tend to have narrow host range except for *M. hapla,* while the mitotic species have a potential host range majorly the higher plants and apomictic species like *M. enterolobii* have restricted host ranges (Trudgill and Blok, 2001). *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* have large and broad host ranges with distinct differences in their overlapping host ranges (Moens *et al*., 2009).

2.4 Incidence and severity associated with root-knot nematodes

Root-knot nematodes are the most damaging nematode pests on vegetable production systems (Potter and Olthof, 1993). They are among the top five most damaging plant pathogens and rank first among the ten most important genera of PPN pests of vegetables (Sasser and Carter, 1985). Anamika *et al*. (2010) reported a disease incidence ranging from 32.8 to 81.6 % and disease severity ranging from 2 to 5 on spinach infected with RKN in India, while Esfahani (2009) and Ravindra *et al*. (2014) reported 100 % disease incidence of RKN in tomato and black pepper in India respectively.

A disease incidence ranging from 5.4 % to 94.4 % was reported in 16 major vegetable areas in Pakistan by Anwar and McKenry (2010) while a RKN incidence ranging from 75-100 % was reported in tomato fields by Shahid *et al*. (2007). Nchore *et al*. (2012b) reported a RKN incidence of 78 % on black nightshade in Kenya. Since there is no single strategy that has been identified to effectively eliminate or suppress RKN population in Kenya, evaluation and

development of cheaper but effective sustainable management strategies is needed for the control of RKN menace on AFNS.

2.5 Identification of root-knot nematodes based on small-sub unit ribosomal DNA

Identification of RKN is a key to disease diagnosis and management on AFNS for vegetable growth, breeding and designing of effective integrated pest management programs (Maleita, 2011). Identification of RKN has been based on female and juvenile morphology (Blok *et al*., 2002), host response (Hartman and Sasser, 1985), isozyme analyses and molecular techniques (Power and Harris, 1993). However, perineal patterns of some *Meloidogyne* species are highly similar, overlapping and require skilled expertise, extensive labour and sometimes inaccurate (Esbenshade and Triantaphyllou, 1990).

Although isozyme analysis is an effective technique to discriminate RKN species, it requires live specimens (Esbenshade and Triantaphyllou, 1985; 1990). Several molecular techniques are valuable tools for identification of RKN species (Hyman, 1996). Enzymatic studies like isozyme phenotypes differentiates major and minor RKN species using species-specific enzyme phenotypes based on polyacrylamide-gel electrophoresis (Esbenshade and Triantaphyllou, 1990). Although such studies have been reported in Kenya on Indigenous leafy vegetables in Kisii and Trans-Mara Counties (Muturi *et al*., 2012), they require live samples and a live reference, hence time consuming.

Identification and characterization of RKN species based on rDNA has been reported in Kenya (Muturi *et al*., 2012). The 5.8S, 18S, 26S, 28S coding genes, internal transcribed spacers (ITS), the external transcribed spacer (ETS) and the

intergenic spacer (IGS) regions are useful in RKN diagnosis and phylogenetic studies due to greater variation in the sequences (Subbotin *et al*., 2013). The small sub-unit (SSU) rDNA gene is conserved region with slowly evolving genes useful in deducing deep phylogenetic relationships among invertebrates (Kjer, 2004; Subbotin and Moens, 2006; Subbotin *et al*., 2013). Characterization of PPN based on 18S rDNA sequence to reveal phylogenetic relationships among nematode populations has been reported in the phylum Nematoda (Blaxter *et al*., 1998; Subbotin and Moens, 2006; Subbotin *et al*., 2013). Moreover, they do not depend on developmental stage of RKN species. Such studies have not been reported on RKN infecting AFNS in Kenya.

Polymerase Chain Reaction (PCR) is a highly sensitive, rapid, accurate and reliable method for DNA amplification and identification that can be applicable on all stages of development in RKN (Power and Harris, 1993; Randig *et al*., 2002). The partial analysis of SSU rDNA sequence among RKN has not been reported on RKN infecting AFNS. The SSU rDNA gene is frequently used to deduce deep phylogenetic relationships among invertebrates (Kjer, 2004). In addition, SSU rDNA is most conserved region among the rRNA encoding genes useful for phylogenetic inference between very distant species (Page and Holmes, 1998; Subbotin *et al*., 2013). Thus, one of the objectives of this study was to identify the RKN species infecting AFNS using single females.

2.6 Management of root-knot nematodes

The damage caused by RKN in most crops is associated with the initial nematode population in soil. In order to manage these nematodes, their initial population has to be reduced (Coyne *et al*., 2009). Management strategies are

therefore, aimed at reducing these initial numbers (McSorley and Gallaher, 1991; Coyne *et al*., 2009). These management strategies can be divided into non-chemical treatments and chemical treatments (Sumbul *et al*., 2015). The non-chemical treatments include among others host plant resistance, organic amendments and soil solarization, while chemical treatment include the use of synthetic nematicides.

2.6.1 Resistant plant varieties

Host plant resistance is among the management strategies used to decrease the risk of damage by many nematode species (Starr and Roberts, 2004). Resistant varieties sustainably suppress and reduce nematode reproduction and populations in the rhizosphere zone (Medina-Filho and Tanksley, 1983; Cook and Starr, 2006; Maleita, 2011). Resistance to plant pathogens is the ability by the plants to lessen or hinder nematode reproduction or overcome attack by plant parasitic nematode (PPN) in relation to a susceptible host (Cook and Starr, 2006; Hussain *et al*., 2014). Plant resistance to PPN is among the most effective and eco-friendly components of integrated nematode management that increases crop yield (Devran *et al*., 2010; Maleita, 2011). Host plant resistance provides an effective, sustainable and economical method for managing PPN in cropping systems (Anwar and McKenry, 2010; Maleita, 2011).

Resistant varieties are compatible to the environment, requires less specialized applications and low inputs (Starr *et al*., 2001; Luc *et al*., 2005; Maleita, 2011). Besides, resistant crops are recommended for fields with high nematode-infestation as they permit growth of susceptible varieties in the rotation schemes (Starr *et al*., 2001; Karssen *et al*., 2013). Studies on host

response to nematode infection have been reported on other crops (Jaiteh, 2010; Sajid *et al*., 2011; Mukhtar *et al*., 2014). Studies on host plant resistance carried out in Kenya on indigenous leafy vegetables reported reduced RKN damage and populations compared to susceptible hosts (Nchore *et al*., 2013). Host resistance has been used to control RKN on tomatoes in Pakistan (Khan, 2009), Ghana (Jaiteh, 2010) as well as tobacco in Kenya (Jogallo, 1984). Such studies on RKN have not been reported on AFNS in Kenya.

The susceptibility of AFNS has great implications on the yield and economic returns. Therefore, information on susceptibility to RKN can be useful to farmers (Khan, 1994). Host plant resistance remains a very important potential component of a solution to many nematode problems of tropical agriculture especially for the low input, small-scale farmers when used in combination with cultural techniques and traditionally grown crops (Luc *et al*., 2005; Maleita, 2011). There is need to screen the available AFNS for resistance or susceptibility to RKN infection under field and greenhouse conditions.

A resistant variety hinders root penetration and development of the parasitic nematodes (Sajid *et al*., 2011). Resistance to nematode damage is an effective tool for improved crop production (Mukhtar *et al*., 2014). Nematode populations are often lower after planting a resistant variety (Cook and Evans, 1987; Mukhtar *et al*., 2014). Resistant cultivars can also be employed as a component of nematode management along with other control strategies like biocontrol (Mukhtar *et al*., 2013a), organic soil amendments (Hussain *et al*., 2011; Kayani *et al*., 2012; Mukhtar *et al*., 2013b), soil solarization (Maleita, 2011), heat treatment and crop rotation with non-hosts for controlling root-knot nematodes. However, a major limiting factor affecting the effectiveness of newly

introduced resistant varieties is the selection of pathotypes or races of nematodes that are able to break down the resistance (Luc *et al*., 2005; Maleita, 2011).

2.6.2 Crop rotation

Crop rotation is one of the cultural methods for controlling plant parasitic nematodes including root-knot nematodes with the aim of reducing the initial population levels of damaging nematode species (Viaene *et al*., 2006). Crop rotation is usually effective when a susceptible host is followed by a poor host after four growing seasons when population densities in soil are low (Bridge, 1996). Besides, growing resistant crop varieties before establishment of AFNS will reduce initial nematode populations in the soil. A study in Western Kenya by Cheruiyot *et al*. (2013) reported the use of resistant crops including *Crotalaria* species and antagonistic crops as rotation crops for management of RKN. Different crops produce different root diffusates that also affect microbial activity and RKN (Viaene *et al*., 2006). Crop rotation is however limited by various factors ranging from diminishing cultivable land, ban of nematicides and occurrence of polyphagous nematode species among other factors that hinders selection of suitable hosts for rotation (Ornat and Sorribas, 2008).

2.6.3 Fallowing and tillage

Root-knot nematodes are obligate phytoparasitic pathogens that require a host plant to complete its life cycle. In absence of the host, the pathogens consume their own reserves leading to starvation and death (Ornat and Sorribas, 2008). High temperature leads to direct soil heating and dessication that

immediately impacts RKN populations. In addition, fallowing and tillage eliminates volunteer plants and weeds as well as reducing soil moisture thus affecting the survival of RKN (Viaene *et al*., 2006). However, this method increases the risk of soil erosion and loss of crop production during fallow period (Viaene *et al*., 2006).

2.6.4 Chemical nematicides

Nematicides are effective in the management of phytopathogenic nematodes either as part of an integrated management program or as the sole control component (Odour-Owino, 2002; Hildalgo-Diaz and Kerry, 2008). The global market for nematicides is about 250,000 tones of active ingredient annually, with vegetables accounting for the greatest proportion of nematicide use and *Meloidogyne* spp. as the target for approximately half of this usage (Haydock *et al*. 2006). However, their use is associated with environmental pollution, toxicicity and un-affordability to small scale farmers (UNEP, 1995). Nematicides are therefore considered to be the last resort in an integrated nematode control strategy (Hasabo and Noweer, 2005; Nchore *et al.* 2012b) and on high value crops. Moreover, most nematicides have been banned and are thus being removed from the market (Hasabo and Noweer, 2005). Sustainable management strategies for RKN pest and diseases are therefore a high priority for the production of healthy AFNS crops.

2.6.5 Organic soil amendments

Organic amendments can be incorporated into the soil to reduce the number and impact of RKN (Muller and Gooch, 1982; Karssen *et al*., 2013) improving soil fertility and structure (Viaene *et al*., 2006; Sumbul *et al*., 2015). Incorporating organic amendments into the soil reduces nematode populations to varying degrees (Karssen *et al*., 2013), increases saprophytic nematodes (Viaene *et al*., 2006; Sumbul *et al*., 2015) and antagonistic organisms in the soil (Akhtar and Malik, 2000; Kimenju *et al*., 2004). Additionally, it leads to the release and consequent build-up of nematicidal compounds like glucosinolates, allelochemicals like antibiotics (Widmer *et al*., 2002; Reddy, 2013), organic acids, phenolic compounds, ammonia or other compounds to concentrations toxic to nematodes (Viaene *et al*., 2006; Timper, 2011; Amulu and Adekunle, 2015; Sumbul *et al*., 2015). Enzymes like collagenase and chitinase that act on the cuticle of nematodes and their eggs are also released (Galper *et al*., 1990).

Organic amendments improved plant growth and productivity due to stimulation of plant growth-promoting bacteria (Widmer *et al.*, 2002; Hassan *et al*., 2010; Sumbul *et al*., 2015). Temperature is increased during decomposition that may inactivate, kill, degrade or render PPN susceptible to other control agents in the soil (Chen and Katan, 1980; Stapleton, 1991; Hasin, 2002).

Soil amendment improves drainage, aeration and water/moisture retention in the soil leading to increased plant tolerance to PPN (Wachira *et al*., 2009; McSorley, 2011). It also increases plant tolerance to nematode infection due to increased abundance of microorganisms active against PPN leading to increased vegetable yields (Kimenju *et al*., 2004; Viaene *et al*., 2006). Korayem (2003)

reported that natural products from plants and crop residue manure reduced PPN population and also improved soil fertility and structure.

The physical, chemical and biological principles of organic soil amendment, as well as its large scale implementation have been researched in many countries around the world (Waceke and Waudo, 2001). Effects of organic soil amendments have been investigated for many vegetable crops such as tomato, chickpea and okra among others (Sumbul *et al*., 2015). Study by Nchore *et al*. (2012a; 2012b) reported that organic amendments from crops, agro-industrial wastes and animal wastes are effective for management of RKN.

The mode of action of organic amendments in the management of PPN involves several mechanisms including stimulation of antagonistic organisms, soil suppressiveness, improves soil fertility and structure and the level of plant resistance to PPN (Stirling, 1991; Korayen, 2003; Viaene *et al*., 2006; Amulu and Adekunle, 2015; Kariuki *et al*., 2015). Application of organic amendments is the most commonly used tactic for enhancing the abundance and activity of antagonists of nematodes (Timper, 2011). Nematode suppression following organic amendments is also attributed to increased saprophytic and antagonistic soil biota (Viaene *et al*., 2006; Kariuki *et al*., 2015).

2.6.5.1 Animal manures

Applying animal manure is a regular practice for improving soil fertility and texture in numerous agricultural systems (Viaene *et al*., 2006). Amendment with animal wastes such as manure has been observed to reduce population of phytopathogenic nematodes in soils (Viaene *et al*., 2006; Nchore *et al*., 2011; Amulu and Adekunle, 2015). Cattle manure is plenty in North Rift and Western

Kenya and is usually heaped in the field (Nchore *et al*., 2011). A tonne of cattle manure contains 5 kg of N, 2.5 kg of P_2O_5 and 5 kg of potash. Application of cattle manure improves soil structure and water holding capacity, water conserving capacity, aeration and biomass. Cattle manure also improves soil salinity and alkalinity. Besides, well decomposed cattle manure contains plenty of micro-nutrients and micro-organisms that are antagonistic to PPN through various mechanisms (Viaene *et al*., 2006). A study in Nigeria by Abubakar *et al*. (2004) revealed that cattle manure effectively reduced RKN population in soil. In Kenya and Egypt, cattle manure has been found to effectively suppress RKN populations in vegetable production systems (Waceke and Waudo, 1993; 2001; Korayem, 2003; Wachira *et al*., 2009; Nchore *et al*., 2012b).

2.6.5.2 *Tithonia diversifolia* compost

The Mexican sunflower (*Tithonia diversifolia*) is a shrub belonging to the family Asteraceae (Palm *et al*., 1996; Gachengo *et al*., 1999). It is widely distributed along farm boundaries in the humid and sub-humid tropics of Africa including Western and Rift valley regions of Kenya (Nyongesa *et al*., 2009; Nchore *et al*., 2012a). *Tithonia diversifolia* compost improves soil fertility as well as soil structure and texture when incorporated into the soil by farmers in Western Kenya for vegetable, maize and banana production (Agbenin, 2004; Nyongesa *et al*., 2009). Green leaf biomass of *T. diversifolia* is high in nutrients, averaging about 3.5 % N, 0.37 % P and 4.1 % K and low content of lignin (6.5%) and polyphenols (1.6%) on a dry matter basis (Jama *et al*., 2000).

Tithonia diversifolia has been reported to have nematicidal properties for nematode management in the production of maize in Western Kenya (Jama *et al*., 2000; Viaene *et al*., 2006). Nchore *et al*. (2011) evaluated the efficacy of *T. diversifolia* compost on RKN in the production of black nightshades in the greenhouse revealing that these organic materials are effective for the management of RKN. Besides, *T. diversifolia* reduced RKN damage in indigenous leafy vegetables in Kenya (Nchore *et al*., 2012a). However, enhancing the efficacy of *T. diversifolia* through solarization for the management of RKN on AFNS has not been reported in Kenya.

2.6.5.3 Agro-industrial wastes

Agro-industrial wastes are an important source of organic amendment at low and affordable cost to resource-poor rural farmers (Nchore *et al*., 2011). Waste crop byproducts, such as sawdust, fruit pulp, coffee husk, oil palm debris and molasses are attractive amendments for nematode management and soil fertility improvement (Viaene *et al*., 2006). They are effective and eco-friendly than chemical nematicides (Ayazpour *et al*., 2010). Use of agro-based industrial residues for the management of PPN has been reported (Viaene *et al*., 2006; Nchore *et al*., 2011; 2012a; Amulu and Adekunle, 2015).

Incorporating agro-industrial wastes into the soil stimulates the release of aldehydes and isothiocyanates, which have biocidal activity and have been related to the control of PPN (Riegel and Noe, 2000). Studies have shown that agricultural and agro-industrial wastes alone and combined with animal manure potentially controls PPN (Piedra-Buena *et al*., 2006). Amending soil with agro-industrial wastes like saw-dust and pymarc is associated with increased populations of

predatory nematodes as well as increasing availability of fungal and bacterial grazing nematodes (Miano, 1999; Korayem, 2003; Viaene *et al*., 2006; Magdoff and Van Es, 2009; Sumbul *et al*., 2015). Wang *et al*. (2002) reported an increase in population of saprophytic, omnivorous and predatory nematodes on soils with low organic matter as well as enhancing nematode-trapping fungi. Tea (*Camellia sinensis*) residues are used to amend soil to reduce RKN population and also to improve soil fertility (Rivera and Aballay, 2008; Nchore *et al*., 2011; 2012a). Therefore, incorporating these materials into soil is not only a possible solution to their disposal but also a feasible way of managing PPN (Nchore *et al*., 2011; Amulu and Adekunle, 2015).

2.6.5.3.1 Pyrethrum marc

Pyrethrum (*Chrysanthemun cinerariaefolium*) marc (Pymarc) is a by-product of pyrethrum with high nutrient concentrations (Nyongesa *et al*., 2009; Nchore *et al*., 2012a). Pymarc is a botanical insecticide that is produced under organic conditions and is known to be highly biodegradable thus making it eco-friendly (Hasabo and Noweer, 2005; Nchore *et al*., 2011). Pymarc is currently used as animal feed in parts of Rift valley and Central Kenya due to its antihelminthic properties (Nchore *et al*., 2011). Additionally, pymarc has been incorporated into maize farming as a nutrient source in Nandi and Western regions (Nyongesa *et al*., 2009).

Pymarc has been reported to reduce RKN populations under greenhouse conditions in Kenya (Miano, 1999; Nchore *et al*., 2012a). Pyrethrum extracts have been reported to have nematicidal properties and have been used for the management of RKN in various countries like Egypt (Hasabo and Noweer,

2005), Indonesia (Wiratno *et al*., 2009), Kenya (Nchore *et al*., 2012a) and Ethiopia (Wondimeneh *et al*., 2013). Enhancing efficacy of pymarc through solarization for the control of RKN has not been evaluated on AFNS in Kenya or elsewhere.

2.6.6 Soil solarization

Solarization is a hydrothermal process accomplished through a combination of physical, chemical and biological mechanisms, compatible with many other soil disinfestation methods (Goswami *et al*., 2013). It involves the placement of plastic sheets on moist soil during periods of high ambient temperature (Elmore *et al*., 1997; Viaene *et al*., 2006; Goswami *et al*., 2013; Karssen *et al*., 2013). Soil solarization for at least 4–6 weeks increases soil temperatures to about 35–50 ºC to depths of up to about 30 cm. Depending on soil type and prior tillage, soil solarization will reduce nematode infestations significantly (Viaene *et al*., 2006; Stevens *et al*., 2003; Reddy, 2013) than the respective temperatures of uncovered wet soil (Kaushika and srivastava, 1980).

Solarization has been used to reduce RKN numbers (Katan *et al*., 1976; Bakr *et al*., 2013; Karssen *et al*., 2013) and other pathogens (Abada *et al*., 2014). Soil solarization is a simple, safe and effective alternative to the toxic and costly chemical nematicides and the lengthy rotation programs needed to control pests and diseases (Katan, 1998; Stevens *et al*., 2003). Thus, soil solarization is an alternative to nematicide for production of AFNS in Kenya.

2.6.7 Integrating organic soil amendment and solarization

Solarization of amended soils has been reported as a technique for controlling *Fusarium* spp., *Pythium* spp. and *Meloidogyne incognita* (Sikora *et al*., 2005; Kaskavalci *et al*., 2007; Klein *et al*., 2011; Abada *et al*., 2014). Solarization has been used effectively to manage RKN in Tanzania (Madulu and Drudgill, 1994), Western Anatolia (Kaskavalci, 2007) and Italy (D`Addabbo *et al*., 2010).

The most important mode of action of solarization biocidal effect has been attributed to direct thermal inactivation of the target pest, heat-induced production of toxic volatile chemicals during decomposition of organic matter and a shift of soil microflora to antagonists of plant pathogens (D`Addabbo *et al*., 2010). Solarization also improves the thermal conductivity and microbial activity of the amended soil (Gamliel *et al*., 2000; Stevens *et al*., 2003).

Effective integration of solarization and organic amendment materials like animal manure, green manure and compost for the control of nematodes and other pests has been reported (D`Addabbo *et al*., 2010; Reddy, 2013). Amended soil reportedly increases temperature in soils beneath the plastic mulch by 37.3 °C. Solarizing soil amended with animal manure and crop residues increases efficacy of nematode pest and weed control (Stevens *et al*., 2003; Abada *et al*., 2014). A study by Oka *et al*. (2007) and Reddy (2013) reported improved root-knot nematode suppression following integration of organic amendments with solarization compared to either treatment alone.

Volatile compounds like ammonia, methanethiol, dimethyl sulfide, allylisothiocyanate, phenylisothiocyanate and aldehyde are released during solarization of organic amended soils and thus augment the biocidal activity of

soil solarization (Gamliel *et al*., 2000; Reddy, 2013). Zasada (2011) reported that solarizing organically amended soil at 26 ^{o}C doubled production of ammonia which is toxic to PPN.

The elevated temperature and toxic gases increases the sensitivity of PPN and other pests to the toxic effect of the retained volatiles (Gamliel *et al*., 2000). Gamliel and Stapleton (1993) reported control of RKN (*M. incognita*) following combination of chicken manure and solarization on lettuce. Such studies have not been carried out in Kenya or elsewhere for the management of RKN on AFNS. One of the objectives of this study was to assess the efficacy of soil solarization on soils amended with selected organic amendments in combating RKN pests on AFNS in Kenya for the first time under field conditions.

CHAPTER 3: MATERIALS AND METHODS

3.1 GENERAL METHODOLOGY

3.1.1 Description of sampling sites

Sampling was carried out in Bungoma County (00^o 34` 00`` N and $034^o$ 34` 00`` E), Kakamega County (00^o 17` 00`` N and $034^o$ 45` 00`` E), Nandi County (034^o 35` 11`` E and $00^o$ 20` 04`` N) and Uasin Gishu (00^o 33` 08`` N and $034^o$ 56` 14`` E)] of Western and North Rift region of Kenya (Figure 3.1) with the potential of year-round growth of AFNS. The study covered eight farm areas located in five agro-ecological zones namely; Marama Central located in Lower midland zone 1 (LM1) in Kakamega County, Chwele in Upper midland zone (UM2), Kibingei (UM2), Mukuyuni (UM1, UM3) and Kimilili (UM2) in Bungoma County, Chepsaita and Osorongai (UM3) in Uasin Gishu County and Chepterwai (UM4) in Nandi County, which are the major African nightshades growing areas in Western and North Rift regions of Kenya.

Bungoma County (Figure 3.1) has biannual rainy seasons with an annual rainfall ranging from 1300 to 1800 mm. Annual temperatures range from 16.4 °C to 29 °C. The wettest season in Bungoma County is experienced between the months of March and July while the driest season comes between October and February. Sampling was carried out in UM1, Coffee zone in UM2 and Coffee-Maize in UM3 agro-ecological zones (AEZs) elevated at an altitude ranging from 1000 m to 1500 m above sea level (a.s.l). The sites are characterized by well drained, deep, dark-red to dark-yellowish-brown friable clay loam (Acrisols), clay (Ferralsols) and sandy clay loam (Arenosols) upland soils with low soil fertility that require to be manured and fertilized every season.

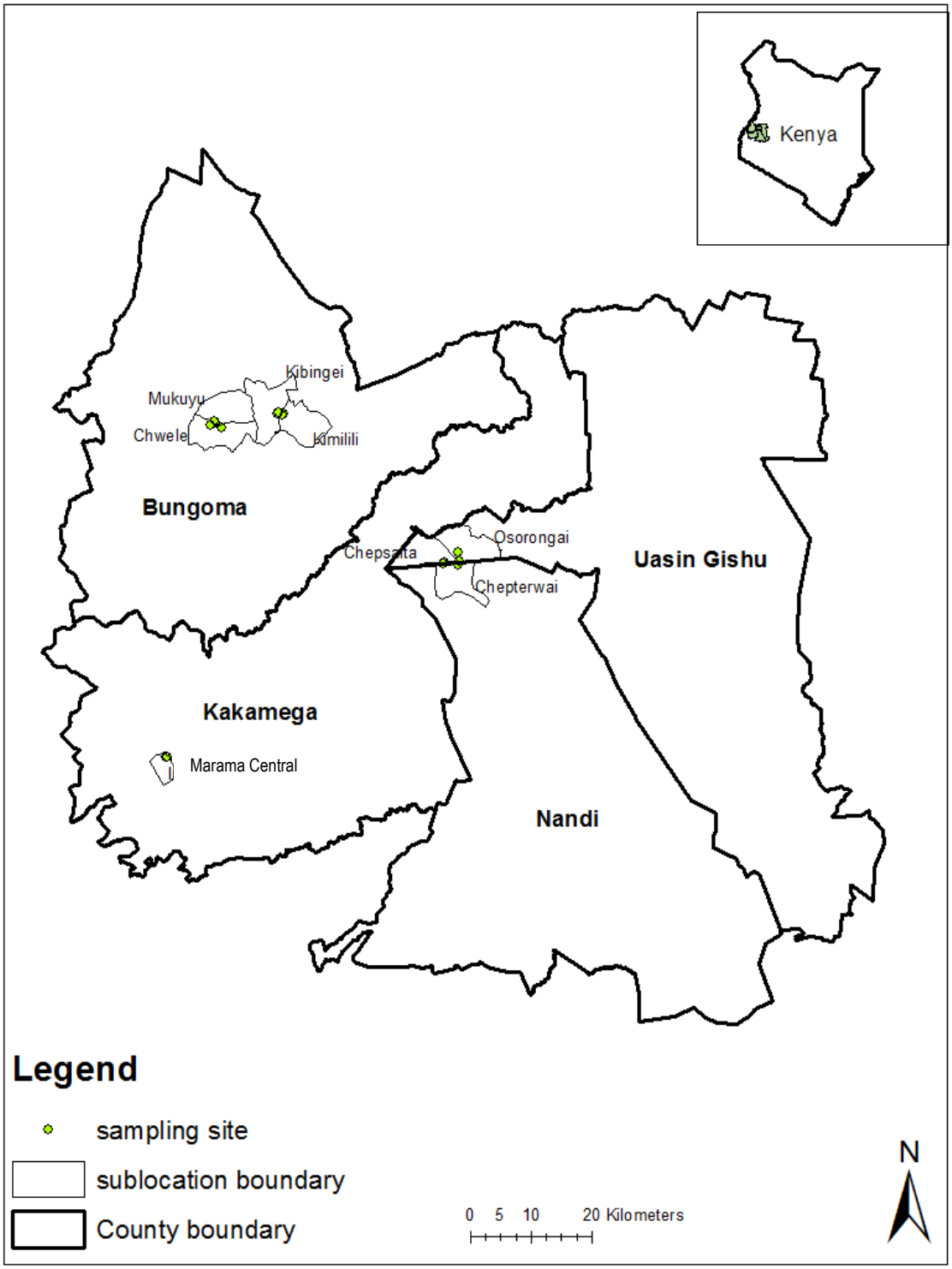

Figure 3.1. Map showing study sites in Western and North Rift region of Kenya (© QGIS 2016 software version 2.10.1-Produced by Nchore).

Uasin Gishu County (Figure 3.1) has two rainy seasons with an annual rainfall ranging from 900 to 1200 mm. The County is situated on a plateau with a cool and temperate climate and annual temperatures range between 8.4 °C and 27 °C. The wettest season in Uasin Gishu County is experienced between the months of April and May while the driest season comes between October and March. Sampling was carried out in Chepsaita (UM3) and Osorongai (Ng'enyilel) located in UM4 agro-ecological zones both elevated at an altitude of between 1500 m – 1900 m level a.s.l. The zones are characterized by well drained, deep, red to yellowish red, friable sandy clay ferralo-chromic acrisols soils with low fertility.

Nandi County (Chepterwai) is characterized with an average annual rainfall of 1358 mm distributed bimodally, March to September long rains and September to December short rains. It experiences mean annual temperature ranging from 17.5 – 19.9 ^{o}C. Sampling was carried out at Chepterwai located in UM4 elevated at an altitude ranging from 1600m to 2000 m. The site has soils with low fertility which overlie murram (Jaetzold and Schmidt, 1983).

Kakamega County (Figure 3.1) is characterized with two rainfall seasons with an annual rainfall ranging from 1800 to 2000 mm with annual temperatures ranging from 21 °C to 22.2 °C. The season is similar to that of Bungoma County. Sampling was carried out in the humid LM1 located at an altitude between 1200 m and 1500 m a.s.l (Jaetzold and Schmidt, 1983). The study site is characterized by well drained, deep to very deep, dark red to yellowish red soils with friable to firm clay acrisols upland soils with low soil fertility that require to be manured and fertilized every season as in Bungoma County.

3.1.2 Sampling procedures

A total of 250 roots and 250 soil samples of African nightshades were collected from farming areas in Chepterwai, Chepsaita, Osorongai (Ng'enyilel), Chwele, Kimilili, Mukuyuni, Kibingei and Marama Central. African nightshades are grown in the aforementioned areas for subsistence and commercial purposes. Rhizosphere soil and root samples were randomly taken from ten different points in each farm. Ten mature AFNS were randomly selected in a zigzag pattern from each farm and dug out gently using a hand trowel, to a depth of 20 cm. The rhizosphere soil adhering to root systems was gently shaken off from the roots, combined and thoroughly but gently mixed before taking a 1 kg of the composite sub-sample. The roots and soil samples were placed separately in polythene bags, sealed and labelled with details of AFNS host, locality and date of collection. The samples were thereafter placed in a cool box and taken to the Kenyatta University Plant Nematology Laboratory where they were refrigerated at 10 ^{o}C until they were processed. Data on the type of the crop, cropping method and irrigation system (if any) were recorded for each farm during the survey using a structured questionnaire (Appendix I).

3.1.3 Source and preparation of organic amendments for solarization experiment

Three months old well decomposed cattle manure was provided by a farmer in Chepterwai. *Tithonia diversifolia* tender shoots were cut from the farm hedges, dried under the shade and manually ground into fine particles. Pymarc was obtained from pyrethrum board of Kenya, Nakuru (Appendix II).

3.1.4 Preparation and treatment of seedbed for screening experiment

Two field sites each measuring 37 × 11 m were cleared, cultivated and ploughed for screening experiment at Kenyatta University (UM3) in Kiambu and Chepterwai (UM4) in Uasin Gishu County. A total of 60 seedbeds with twenty seedbeds per block each measuring 1.2 × 3.2 m were prepared for each site. The sites were naturally infested with RKN. Plots without nematodes received 200 ml ha^{-1} (0.078 ml $plot^{-1}$) of Real Trichoderma®.

3.1.5 Field preparation for solarization experiment

The field site for solarization at Chepterwai was cleared of weeds, leveled and thereafter debris and large soil clods were removed. A total of 48 plots each measuring 2.4 × 6.4 m with a 1 m guard row between the plots, replicated three times (16 plots per block) in RCBD were prepared. The plots were amended with organic amendments (OAs) at the rates of 4 t ha^{-1} for *T. diversifolia* and 5 t ha^{-1} for pymarc and cattle manure respectively and immediately the field was watered to 20 cm soil depth. Plots without nematodes received 200 ml ha^{-1} of Real Trichoderma®. Solarized plots were covered with clear and transparent polythene mulch. Unamended and non-solarized plots served as the control.

3.1.6 Physicochemical and mineral content analysis of soil samples and organic amendment materials

Five hundred (500) grams of soil composite and each organic amendment were taken to the Kenya Agricultural and Livestock Research Organization-National Agricultural Research Laboratories (KALRO-NARL) for chemical and mineral content analyses. The physicochemical analysis of soil and mineral

content analysis of cattle manure, *T. diversifolia* compost and Pymarc amendments before the experiments and treated soil at the end of experiments were carried out as follows: $N\text{-}NH_4^+$ using a modified Nessler's colorimetric method (Wu and Cao, 2013); $N\text{-}NO_3^-$ by colorimetric method with phenol- 2,4 disulphonic acid, $N_{anorg.}$ was calculated as a sum of $N\text{-}NH_4^+$ + $N\text{-}NO_3^-$. The content of P was determined by colorimetric method (Fogg and Wilkinson, 1958; Mehlich, 1984), K and Ca by flame photometry (Dean, 1960; Mehlich, 1984), Mg by atomic absorption spectrophotometry (Orlov and Grisina, 1985; Welz and Sperling, 1999), pH_{H20} and pH_{KCl} (in solution of 1.0 mol KCl dm^{-3}) potentiometrically.

The total N content (N_t) was determined according to Kjeldahl method (Bremner, 1960), while total carbon content (C_{ox}) was determined spectrophotometrically after oxidation using T˘ jurin method (Orlov and Grisina, 1985).

3.1.7 Data collection on plant growth parameters

Data on the height, dry shoot weight and fresh root biomass and disease parameters for the AFNS were collected both in the greenhouse and field experiments sixty (60) days after inoculation with RKN. Plant height was measured from the soil line to the shoot apex with a cotton thread at the termination of greenhouse and field experiments. For easier removal of plants from the soil, the sides of the pot were pressed to loosen the soil, while in the field a soil auger was used. Soil was removed from roots by gently shaking the plants according to the procedure described by Ateeq-ur-Rehman (2009). Roots were separated from stems, washed under running tap water and dabbed dry

with tissue paper. Fresh root biomass of each plant was measured using Leica electronic balance by placing the dabbed root system on the scale and taking the readings.

The stem of each plant was cut into 5-cm pieces and placed in 1 kg size khaki sample paper. Thereafter, the shoots were oven dried at 70 ^{o}C for 3 days (72 hours) until a constant dry weight was obtained. The weight of an empty khaki paper was also determined after oven drying.

3.1.8 Assessment of root-knot nematode disease parameters

Root systems of African nightshades (AFNS) from both greenhouse and field experiments were harvested at the end of the experiment, washed separately and dabbed dry with a tissue paper to assess galling index (GI), egg-mass index (EMI), J2 populations and reproduction factor. The GI was scored on scale of 0-10 rating chart by Bridge and Page (1980) where; 0 = no galls, 5 = 50 % of the roots infected and 10 = entire root system galled and plants usually dead (Appendix II).

A five grams root sub-sample of AFNS was immersed in Phloxine B solution as described by Holbrook *et al*. (1983) to stain the egg-masses. Stained roots were rinsed with running tap water and dabbed dry. Egg-masses were visualized on a stereo microscope and manually enumerated with a Laboratory Counter and scored using a 0-5 egg-mass rating index according to Quesenberry *et al*. (1989) where; 0= no egg-masses; 1= 1-2; 2= 3-10; 3= 11-30; 4= 31-100 and 5= > 100 egg-masses per root system.

The root system was cut into small pieces of 1 cm long using a pair of scissors. Five grams of each plant was blended for 10 seconds and thereafter placed in a plastic sieve lined with a ply-two tissue paper placed in a plastic plate. Tap water was poured gently into the plastic plate in which the sieve was placed until the tissue became moist. The set-up was left for 48 hours and the contents in the plates were poured out separately into beakers and left overnight for the juveniles to settle (Thomas, 1959). Each nematode suspension was adjusted to 20 ml for standardization and J2s counted using a Leica Ms5 stereo microscope at 40 × magnifications and enumerated with a hand counter.

The soil was thoroughly but gently mixed and a 100 cm^3 of the soil was used for assessing nematode population. The soil was placed on a double layer of serviette tissue paper supported by a plastic sieve and placed over a shallow extraction tray. Water was gently added to the tray until the soil was just wet and thereafter the set-up left for 48 hours. The nematodes were extracted by pouring the contents of the tray into a beaker. Thereafter, the J2s suspension was left overnight and excess water siphoned gently with a pipette into a beaker. The excess water was poured through a 38 µm sieve and then backwashed with water from a wash bottle onto a 50 ml beaker (Thomas, 1959) to recorver and J2 that might have been siphoned. The J2s were counted and enumerated as described earlier.

The nematode reproduction factor was calculated as R = Pf/Pi, where Pf = final J2 population and Pi = 3 000 J2s pot^{-1} in the greenhouse or J2 populations in the field experiments. Significant differences between treatments were determined by ANOVA procedures at a probability level of 5 %.

Host status was based on Rf according to Zhang and Schmitt (1994) where; Immune (I) if Rf = 0; resistant (R) if 1 > Rf > 0; tolerant (T) if 5 ≥ Rf > 1 and susceptible (S) if Rf > 5.

Root-knot disease severity was rated using the following key based on the galling index developed by Taylor and Sasser (1978).

Severity scale	**Disease intensity**
0	Disease free
1-2	Very mild
3	Mild
4-5	Moderate
6-8	Severe
9-10	Very severe

3.2 SPECIFIC METHODOLOGY

3.2.1 Knowledge, awareness and management of root-knot nematodes on African nightshades

A survey study was carried out on selected farms in Chepterwai and Ng'enyilel in Uasin Gishu County, Bituyu, Kibingei, Chwele and Mukuyuni in Bungoma County and Marama Central in Kakamega County to assess farmers' knowledge, awareness and management of RKN on AFNS in June 2014 using a structured questionnaire (Appendix I). Thirty farmers were randomly selected (ten from each County) from different farm areas in Uasin Gishu, Bungoma and Kakamega Counties for interview and discussions. The selected areas were among the major African nightshade producing areas in Kenya (Mbogoh *et al*., 2013).

The sample size of 30 farmers was determined according to Mugenda and Mugenda (1999). The formula used is shown below;

$$nf = \frac{n}{1+\frac{n}{N}}$$

Where; nf = the sample size, n = a constant (384) and N = 33 farmers.

Interviews and discussion were carried out in English for the three Counties. In addition, farmers' practices on production of AFNS were also assessed to determine their impact on RKN damage on AFNS.

The responses to questions on age, educational background, vegetable production constraints, farm and pest management practices were recorded. For each question, the percentage of farmers who gave similar responses was calculated for each locality.

Data on the farmers' literacy were collected based on the level of schooling and the lengthy of time spent in school. Farmers who had primary education were regarded as having basic education, while those with secondary education were treated as having formal education. Farmers with neither basic nor formal education (did not attend school) were categorized as illiterate.

3.2.2 Root-knot nematode disease incidence and severity on African nightshade in selected Counties of Kenya

A root-knot nematode survey was carried out on selected farms in Chepterwai and Ng'enyilel in Uasin Gishu County, Bituyu, Kibingei, Chwele and Mukuyuni in Bungoma County and Marama Central in Kakamega County described in section 3.1.1 to determine RKN disease incidence and severity on AFNS. The rhizosphere soil and root systems were taken from depths of 20 cm using a hand shovel from ten different points from each farm while moving randomly in a zig-zag pattern. To avoid contamination and spread of nematode pathogens, the shovel was cleaned before moving to the next farm.

Nematode sampling was carried out as described in section 3.1.2 and the roots were processed for nematode galling according to Bridge and Page (1980) as described in section 3.1.8. The severity of RKN infection was rated using the key developed by Taylor and Sasser (1978) based on the galling index as described in section 3.1.8.

Disease incidence was detected in each farm through visual examination of the root system for root-knot galls and expressed according to a method by Nchore *et al*. (2012a) using the formula;

$$\text{Root} - \text{knot nematode incidence} = \frac{\text{Number of AFNS plants with galls}}{\text{Total number of AFNS plants sampled}} \times 100$$

3.2.3 Identification of root-knot nematodes on African nightshades

The RKN (*Meloidogyne* species) were characterized using an optimized protocol for partial analysis of SSU rDNA sequences according to a procedure by Holterman *et al*. (2006).

3.2.3.1 Isolation and amplification of root-knot nematode DNA

The genomic DNA was isolated from female nematodes according to a protocol by Holterman *et al*. (2006). The mastermix for amplification (PCR) reaction of the DNA was carried out using 25.05 µl reaction mixture prepared as shown in Appendix IV.

Sixteen DNA samples and a control were used for PCR reactions. The DNA template and master mix solution was placed in PCR strips while the control was placed in single PCR tubes. For a single PCR reaction, 23.75 µl of the master mix was pipetted into each tube before adding 1.25 µl of DNA template from a single RKN female. In addition, 1.25 µl of water was included as negative control and 1 kb ladder (Bioline Ltd) as reference. The SSU rDNA was amplified as 2 partially overlapping fragments using 988F/1912R, 1096F/1912R and 1813F/2646R primer pairs with 1912R being a specific primer (Figure 3.2 and Appendix IV).

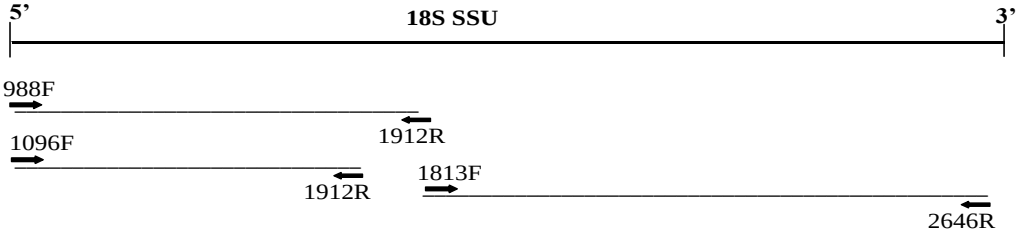

Figure 3.2: Sketch diagram showing prime positions for PCR.

The PCR master mix was mixed by flipping the tubes gently followed by spinning down the PCR samples in the mini centrifuge. The samples were placed in the programmable PCR Thermocycler machine (Biometra®) and an appropriate program and PCR conditions set (Appendices VI – VIII).

3.2.3.2 Agarose gel electrophoresis

A 1 % agarose gel was prepared using 1× TBE-buffer and stained with 4 µl 1:4 Serva G according to Holterman *et al*. (2006). The first well was loaded with 3.5 µl 1 kb marker, followed by 7 µl of each amplified product and a control. The amplified products were run at 90 volts for 30 minutes and DNA bands were visualized on UV light. The images were photographed using a Biometra® camera according to Holterman *et al*. (2006).

3.2.3.2.1 Purification and sequencing of PCR products

Purification of amplified products was performed using 20 micro liters (µl) of the PCR products and a co-precipitant according to a modified protocol by Holterman *et al*. (2006). An equal volume of SureClean® was added and mixed thoroughly, followed by incubation at 18 ^{o}C for 10 minutes. The mixture was centrifuged at a maximum speed (13200 rpm) for 10 minutes and the supernatant was removed carefully by aspiration. A double volume (40 µl) of 70 % ethanol to the original sample volume was added, vortexed for 10 seconds, centrifuged at maximum speed (13200 rpm) for 10 minutes followed by removal of the supernatant. The contents in the tube were centrifuged under speed vacuum for 5 minutes at the highest drying rate to remove ethanol

completely. The DNA was re-suspended in 20 µl MilliQ water and its concentration in ng/µl measured at 260 nm via Nanodrop with MilliQ water used as a blank. The DNA concentration was recorded (Appendix IX).

The samples for DNA sequencing were marked on the top with consecutive numbers according to the farms. Ten (10) pmol/µl primer aliquots in individual tubes were sent together with the samples to Macrogen Biotech Company in Netherlands for sequencing.

The amplified PCR products were sequenced directly and the sequences assembled using Virtual box, Biolinux7. The DNA sequences were inspected manually using biological sequence alignment editor (BioEdit 7.2) and Staden package (PreGap4 and Gap4 programs) to generate a single consensus DNA sequence of 1.6 kb and analysed with MEGABLAST program for phylogenetic relationship between RKN isolates.

3.2.4 Response of African nightshades to root-knot nematodes inoculation

Nine African nightshades (AFNS) including six improved species sourced from The World Vegetable Centre (AVRDC) and three landraces from farmers in Kisii and Kakamega in Western Kenya were screened for their response to mixed population of RKN in the greenhouse and field conditions. The AFNS that were screened were; *Solanum villosum* (Sv) line BG03, *S. nigrum* (Sn) line IP03, *S. eldoretianum* (Se) line MW05, *S. scabrum* (Ssc) line Rv1, *S. americanum* (Sa) line RC01 and *S. sarrachoides* (Ssa) line MW13 sourced from AVRDC, *S. nigrum* complex (Snc) landrace from Kakamega, *S. opacum* (So) landrace from Kisii and *S. nigrum* from Simlaw Seeds Company (Sns). Tomato

(*Solanum lycopersicum* cv. money-maker) purchased from Kenya Seed Company was included as a positive control.

Seeds were raised in sterilized sand: soil (2: 1) mixture in seedling trays in the greenhouse at Kenyatta University at a temperature of 24 ± 3 ^{o}C. After germination, seedlings were thinned and allowed to grow for three weeks after which a single seedling was transplanted into a 12 cm-diameter pot. The pots were inoculated with 3 000 J2s of mixed RKN population and arranged in RCBD with four replications each. The experiment was terminated 60 days after inoculation. Data on plant growth and disease parameters were carried out as described in sections 3.1.7 and 3.1.8. All AFNS that were resistant and tolerant to RKN in the greenhouse experiment were screened under the field conditions to determine the best species.

In the field experiment, 60 plots were prepared as described in section 3.1.4. Thereafter, initial J2 populations on each plot were determined through soil sampling. The control plots were treated with 200 ml ha^{-1} (0.078 ml $plot^{-1}$) of Real Trichoderma®. Three seeds of each AFNS were planted per depression. Di-ammonium phosphate (DAP) fertilizer was used at the rate of 120 g per plot (300 kg ha^{-1}) during planting. Two weeks after germination, the seedlings were thinned to one plant per depression with each plot having 10 rows and 6 plant units per row at a spacing of 15 × 30 cm giving a total of 60 plants per plot (156,250 plants per hectare equivalent). Mechanical weeding was carried out four weeks after transplanting to ensure the plots were free from weeds. Five plants and soil samples from each of the plots were selected randomly, uprooted gently and data on plant growth and disease parameters obtained at termination of experiment as described in sections 3.1.7 and 3.1.8. Percentage reductions or

increase in shoot height, dry shoot weight and fresh root weight were calculated over the controls as described by Irshad *et al*. (2012) as follows;

$$\text{Percentage reduction or increase} = \frac{\text{Uninoculated} - \text{inoculated}}{\text{Uninoculated}} \times 100$$

3.2.5 Efficacy of solarizing soils amended with selected organic materials on root-knot nematodes on African nightshades

A field experiment to integrate solarization with *T. diversifolia*, cattle manure and pymarc was carried out during the hot and dry season (October 2014 – March 2015) in a farmer's field that was infested with RKN in Chepterwai, Uasin Gishu County (00^{o} 34` 03N and $034^{o}$ 57` 53E at 1806 m a.s.l). The field as described in 3.1.5 was measured into 48 micro-plots each measuring 2.4 × 6.4 m replicated three times with 1 m spacing between the blocks. The soil in the study site was loam with a pH of 5.32. Soil samples were taken before initiation of the experiment, at termination of solarization and at the end of the experiment. Six soil cores at 5 cm and 15 cm depth were randomly collected from each plot, composited into 1 kg sample for each depth and transported to the Kenyatta University Plant Nematology Laboratory. Nematodes were extracted and enumerated as described in section 3.1.8. Five hundred grams (500 g) soil sub-samples and each organic material were taken to the Kenya Agricultural and Livestock Research Organization National Agricultural Research Laboratories (KARLO-NARL) Kenya, for physicochemical and mineral content analysis respectively.

The experimental design for each experiment was a 2 × 2 × 4 factorial, with two solarization treatments (solarized or control), two nematode levels (with or without) and four soil amendment (Cattle manure, Pymarc, *Tithonia diversifolia*

and unamended control) treatments. The treatments used were; Cattle manure (Cm) + Nematode (N); Pymarc (Pm) + N; *Tithonia diversifolia* (Td) + N; Unamended soil (NOA) + N; Cm – N; Pm – N; Td – N and NOA – N. The individual plots received cattle manure at the rate of 7.68 kg $plot^{-1}$ (5 t ha^{-1}), pymarc at the rate of 7.68 kg $plot^{-1}$ (5 t ha^{-1}) and *T. diversifolia* at the rate of 6.144 kg $plot^{-1}$ (4 t ha^{-1}). The control plots were not amended. Plots without nematodes received 0.31 ml $plot^{-1}$ (200 ml ha^{-1}) of Real Trichoderma®.

The plots were watered to 20 cm depth and covered with a single layer of 25-µm thick, clear, low-density polythene mulch for solarization. The edges of the polythene mulch were buried into a trench around the treated plots to prevent wind from blowing or tearing it. Out of all the treatments undertaken, one set was covered with clear transparent polythene mulch while the other set was not covered and was therefore unsolarized. Non-amended plots without polythene mulch served as absolute controls. Treatments were replicated 3 times in a randomized complete-block design in plot measuring 2.4 × 6.4 m as described in section 3.1.5. The polythene mulch was left for 5 weeks allowing the soil to heat to the greatest depth possible. The soil temperature of the solarized and non-solarized plots was recorded at depths of 5 and 15 cm at an interval of five days using a soil thermometer.

At the end of five weeks, the polythene mulch was removed and the soil was allowed to dry to workable texture. Immediately after terminating solarization, samples (500 g) of treated soil were obtained from 5 cm and 15 cm depths from ten points on each treated plot. Shallow cultivation was carried out to prevent bringing up pathogens from the lower levels of the soil. The percentage

nematode control for each treatment at 5 cm and 15 cm was calculated as follows;

$$\text{Percentage control} = 100 - \left(\frac{\text{J2 population on solarized plot}}{\text{J2 population on unsolarized plot}} \times 100\right)$$

Three seeds of *Solanum villosum* were planted and covered lightly with soil at a depth of 1 cm per depression in ten rows at spacing of 15 cm x 30 cm. Two weeks after germination, the seedlings were thinned to one seedling per depression with each row having six seedlings. The data on plant growth and disease parameters were determined as described in section 3.1.8.

3.3 Data analysis

Data from the questionnaire were analyzed using Pearson Chi-square while frequencies and percentage variable occurrences were calculated using cross tabulation (PROC FREQ), in IBM SPSS statistics 20. Data for disease parameters were subjected to Analysis of Variance (ANOVA) using SAS portable (Version 9.1.3) and MS Excel computer programs. Prior to statistical analyses, data were checked for normality and transformed where necessary. A logarithmic transformation (Log_{10} [x+1] where x = nematode populations per 100 cm^3 soil or egg-masses or galls or their indices in a single sample) was applied to the data on nematodes and then subjected to one way Analysis of Variance (ANOVA). Where ANOVA indicated a significant treatment difference, the Least Significant Difference (LSD) at 5 % was used to separate the means. Untransformed means were presented in tables and charts.

Data from shoot height, shoot weight and root weight of AFNS for the screening experiment were subjected to two sample t-test analysis to compare the treatments or to two-way ANOVA for the solarization experiment to test for main treatment effects using SAS portable (Version 9.1.3) and Minitab (Version 15) computer programs. Correlation was performed to determine the relationship between RKN galling index/Rf/J2 populations with dry shoot weight and fresh root weight.

Pairwise and multiple sequence alignment against the 18S rDNA sequences alignment (NCBI ref AB905314.1; AY268119.1; AY593892.1; AY942621.1; FJ559408.1; JQ768373.1; JX100420.1; AY268121.1; KC545968.1; KF993644.1; KJ636268.1 and KJ641552.1) and AF442190.1, AF202164.2 and KJ869413.1 as outgroups from GenBank were performed using ClustalW on Molecular Evolutionary Genetic Analysis (MEGA6 Version 6.06) computer program according to Thompson *et al*. (1994).

Phylogenetic relationship among the RKN species was determined on Maximum likelihood aligorithms using MEGA 6 (Tamura *et al*., 2004; 2013). Evolutionary divergence between the isolates was conducted in MEGA6 with Maximum Composite Likelihood Model according to Tamura *et al*. (2013), while the rate and pattern of nucleotide substitution was estimated with Tamura-Nei model. Nucleotide diversity for the aligned sequences for the RKN isolates from the surveyed region was determined using Tajima's Neutrality Test.

CHAPTER 4: RESULTS

4.1 Impact of farmers' knowledge, awareness and management on RKN damage on African nightshades

The type of AFNS grown by farmers differed significantly ($\chi^2 = 23.700$, $P < 0.05$) with majority of the farmers growing traditional type of AFNS compared with those who grew improved types of AFNS or both types as shown in Table 4.1. All the AEZs had farmers who grew traditional types of AFNS with UM3, followed by UM4 and UM2 having the highest proportion, and UM1 and LM1 had the least. On the other hand, improved AFNS type was only grown in LM1 (Table 4.1).

Table 4.1: Varieties of African nightshade grown by farmers in the sampled Agro-ecological zones

Type of African nightshade	Agro-ecological zones (AEZs)					
	LM1	UM1	UM2	UM3	UM4	Mean (%)
Traditional	3.33	6.67	13.33	26.67	16.67	66.67
Improved/Agriculture type	20.0	0.0	0.0	0.0	0.0	20.0
Both	10.0	0.0	3.33	0.0	0.0	13.33
Total within Counties	33.33	6.67	16.66	26.67	16.67	100.0
Pearson Chi-square	($\chi^2 = 23.700$, P = 0.003)					

The study revealed that 66.7 % of the farmers grew AFNS using their own seeds preserved from the previous harvest, 23.3 % used certified seeds and 6.7 % obtained seedlings from their neighbours or from the market (3.3 %) as indicated in Table 4.2. The AFNS crops were established in rows or lines system for commercial (40.0 %), subsistence (16.7 %) or both (43.3 %).

Table 4.2: Source of African nightshade seedlings grown by farmers in the sampled Agro-ecological zones

Source of AFNS seedlings	Agro-ecological zones (AEZs)					
	LM1	UM1	UM2	UM3	UM4	Mean (%)
Certified	20.0	0.0	0.0	3.33	0.0	23.33
Own	10.0	6.67	10.0	23.33	16.67	66.67
Neighbours	3.33	0.0	3.33	0.0	0.0	6.66
Market	0.0	0.0	3.33	0.0	0.0	3.33
Pearson Chi-square	(χ^2 = 20.202, P = 0.063)					

Although crops grown before establishing AFNS did not differ significantly (χ^2 = 42.011, P = 0.557) between the AEZs, most farmers grew maize (36.7 %) or cabbage (10.0 %) before AFNS, while 6.7 % of the farmers rotated beans with AFNS. Farmers also grew AFNS on land previously grown with groundnuts, potatoes, *Capsicum*, tomatoes or intercropped with either tomatoes, kales or beans while others used virgin land or fallow land (Figure 4.1).

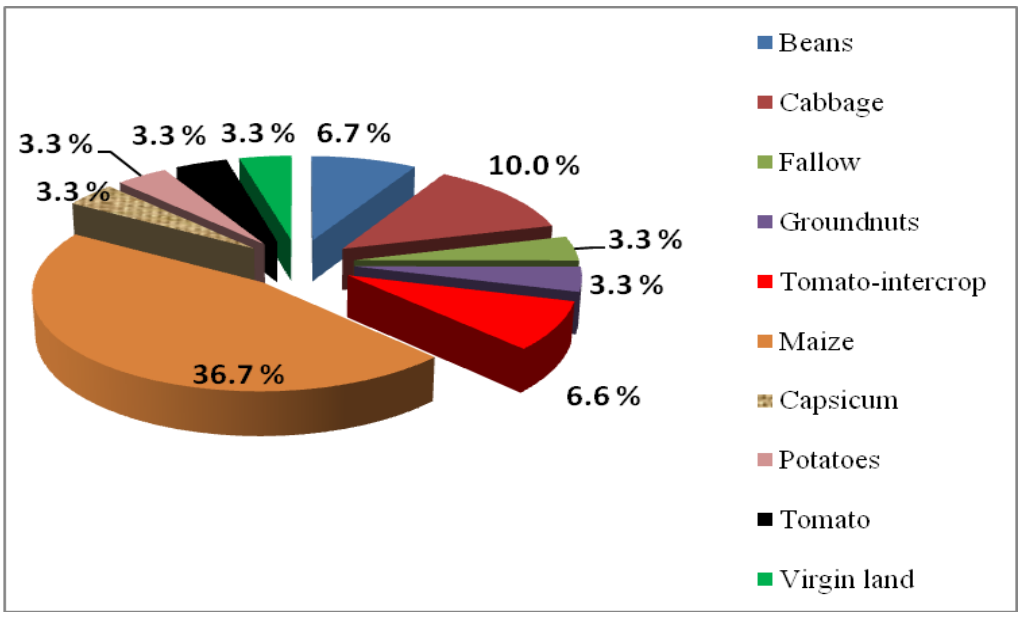

Figure 4.1: Proportion of crops grown by farmers before or intercropped with African nightshades.

In absence of rainfall, 53.3 % of the farmers irrigated AFNS crops while 46.7 % of them did not (Appendix X). The source of water differed significantly (χ^2 = 36.509, P < 0.05) between the AEZs. Of the farmers irrigating AFNS, 40.0 % sourced their water from river with LM1 having the largest proportion, followed by UM2 and UM3 with 6.67 % and 3.33 % respectively (Appendix X). A small proportion (3.33 % each) of the farmer sourced water from the stream, bore holes, piped water and wells respectively (Appendix X).

The source of organic and inorganic materials for establishing AFNS did not differ significantly (χ^2 = 27.044, P > 0.05) across the AEZs (Table 4.3). The study reported that 66.7 % of the farmers applied organic sources of manure in addition to inorganic fertilizers during planting of AFNS with 33.3 % depending solely on Di-ammonium phosphate (DAP) fertilizers. Of the organic materials, cow manure, followed by farm yard manure and compost manure were applied by a large proportion of farmers, while chicken waste, sheep and goat, and sheep and cow manures were applied by 3.33 % of the farmers respectively (Table 4.3).

Table 4.3: Proportion of farmers using organic manure and fertilizer for growth of African nightshades

	Agro-ecological zones					
Organic manure	LM1	UM1	UM2	UM3	UM4	%
Cow manure	0.0	3.33	10.0	10.0	3.33	26.67
Chicken waste	3.33	0.0	0.0	0.0	0.0	3.33
Sheep and goat manure	0.0	0.0	0.0	3.33	0.0	3.33
Sheep and cow manure	0.0	0.0	0.0	0.0	3.33	3.33
Compost	10.0	0.0	0.0	0.0	0.0	10.0
Farm yard manure	13.33	0.0	3.33	0.0	3.33	20.0
DAP fertilizer	6.67	3.33	3.33	13.33	6.67	33.3
Pearson Chi-square	(χ^2 = 27.044, P = 0.302)					

4.1.1 Social economic characteristics of the respondent farmers

Although majority of the respondents in the study area were male (76.7 %) compared with the female (23.3 %) there was no significant difference (χ^2 = 7.081, P > 0.05) established between the sampled AEZs. The male farmers were highest in LM1 and UM3 (26.67 %) respectively, followed by UM4 (13.33 %) and UM1 (3.33 %) that had the lowest proportion.

The age of the farmers did not differ significantly (χ^2 = 9.595, P > 0.05 between the various AEZs. The sampled farmers were adults in the ages of 20 – 66 years with mean age of 38.68 ± 2.587 years and 20.0 % of the farmers aged between 26 – 30 years.

4.1.2 Education level of farmers and growth of African nightshades

Farmers' education level differed significantly (χ^2 = 9.456, P < 0.05) among the AEZs. Majority (93.3 %) of the sampled farmers were literate with only a few of them (6.7 %) being illiterate. A large proportion (73.3 %) of the literate farmers had primary education while 20.0 % of the farmers had secondary education. The study revealed that 86.6 % of the farmers were fully dedicated to farming with only a small proportion involved in other activities.

4.1.3 Farmers' awareness of root-knot nematode on African nightshades

The farmers' ability to recognise RKN on African nightshades (AFNS) did not differ significantly (χ^2 = 3.697, P > 0.05) with their level of education but the length of time they have been growing AFNS. Whereas farmers had good knowledge of arthropod pests, insects and diseases of AFNS, RKN pests were

unknown to most (53.6 %) farmers except for a few (46.4 %) who identified RKN problem based largely on the type of damage or symptoms. Of the farmers that were aware of RKN, majority (54.5 %) had primary school education compared with those that had secondary education (16.7 %). In addition, it was noted that more male (73.3 %) compared to female (26.7 %) farmers knew RKN pests.

The length of cultivating AFNS did not differ significantly ($\chi^2 = 6.404$, $P > 0.05$) with the farmers level of education. Farmers who had primary education had grown AFNS for 34 years compared with their counterparts with secondary education who had grown AFNS for 10 years.

Farmers' knowledge on RKN damage on AFNS differed significantly ($\chi^2 = 17.300$, $P < 0.05$) between the AEZs with all the farmers in LM1 being aware of the RKN (100 %), followed by UM1 (50 %), UM3 (37.5 %) and UM2 (20 %), while none of the interviewd farmers in UM4 was aware of RKN (Figure 4.2).

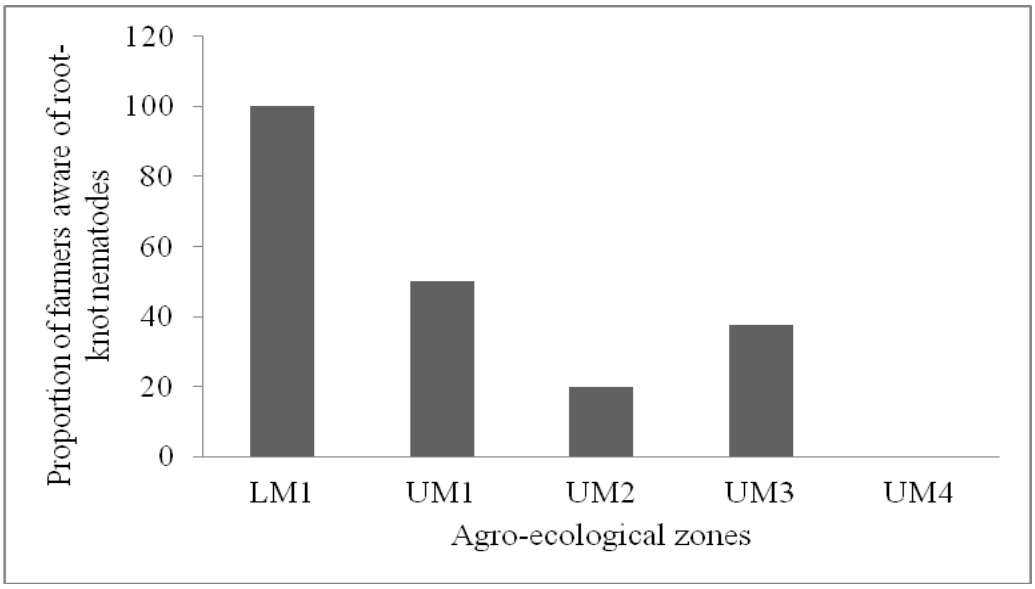

Figure 4.2: Proportion of farmers aware of root-knot nematode in the surveyed Counties.

The ability of the farmers to identify the symptoms caused by RKN damage on AFNS differed significantly (χ^2 = 40.025, P < 0.05) between the various AEZs (Table 4.4). A large proportion of the farmers (50.0 %) could not identify symptoms caused by the RKN pests on AFNS in their farms (Table 4.4). Of these farmers, 16.67 % were in UM3 and UM4 respectively, while UM2 and UM1 had 13.33 % and 3.33 % respectively (Table 4.4). Symptoms related to RKN infection on the crops including yellowing or chlorosis of plants, wilting, swollen roots and short stems were notably mentioned by farmers (Table 4.4).

Table 4.4: Ability by the farmers to identify symptoms associated with RKN on AFNS in the various Counties

Symptoms associated with RKN on AFNS	**Agro-ecological zones**					
	LM1	UM1	UM2	UM3	UM4	Mean%
Do not know symptoms	0.0	3.33	13.33	16.67	16.67	50.0
Swellings on roots	0.0	3.33	0.0	0.0	0.0	3.33
Short stems	0.0	0.0	3.33	0.0	0.0	3.33
Yellowing of leaves	30.0	0.0	0.0	0.0	0.0	30.0
Wilting and drying	3.33	0.0	0.0	0.0	0.0	3.33
Pearson Chi-square	(χ^2 = 40.025, P = 0.001)					

4.1.4 Influence of pest management practices on root-knot nematode damage on African nightshades

The control practices employed by farmers to curb nematode pests differed significantly (χ^2 = 42.234, P < 0.05) between the AEZs (Table 4.5). Most farmers (53.33 %) did not control nematode pests on their farms because they did not know the causal agent or the appropriate management practice. However, 26.6 % of the farmers used pesticides like Diazinol® and Duduthrin®

ment for controlling arthropod pest hoping to combat nematode pests with a large proportion being from LM1 (Table 4.5). A small proportion (3.33 % each) of the farmers were also using non-chemical practices like crop rotation, uprooting infected crops and application of woodash or cutting stems of diseased crops to combat the nematode pests (Table 4.5). On the other hand, 3.33 % of the farmers in UM3 used nematicides like Mocap® to combat nematodes (Table 4.5).

Table 4.5: Proportion of root-knot nematode management practices by farmers in the surveyed Agro-ecological zones

Root-knot nematode management practices	**Agro-ecological zones**					
	LM1	UM1	UM2	UM3	UM4	Mean %
No control	0.0	6.67	13.33	16.67	16.67	53.33
Crop rotation	0.0	0.0	0.0	3.33	0.0	3.33
Pesticides	23.33	0.0	0.0	3.33	0.0	26.66
Uprooting infected crops	3.3	0.0	0.0	0.0	0.0	3.33
Mocap nematicide	0.0	0.0	0.0	3.33	0.0	3.33
Cutting infected crops	0.0	0.0	3.33	0.0	0.0	3.33
Woodash	0.0	0.0	0.0	0.0	0.0	6.67
Pearson Chi-square	(χ^2 = 42.234, P = 0.041)					

4.2 Root-knot nematode incidence and disease severity on African nightshades

4.2.1 Root-knot nematode disease incidence

All the farms were infested with root-knot nematodes with an average disease incidence of 94.13 % (Figure 4.3). The disease incidence did not differ significantly ($P > 0.05$) between the agro-ecological zones (AEZs) as indicated in Figure 4.3. The highest disease incidence was recorded in UM3 (98.75 %), followed by UM2 (96.0 %), UM4 (94.0 %) and UMUM1 (90.0 %) while the lowest disease incidence (86.0 %) compared with the other AEZs was recorded in LM1 where farmers grew the giant nightshade which is the improved line of AFNS (Figure 4.3).

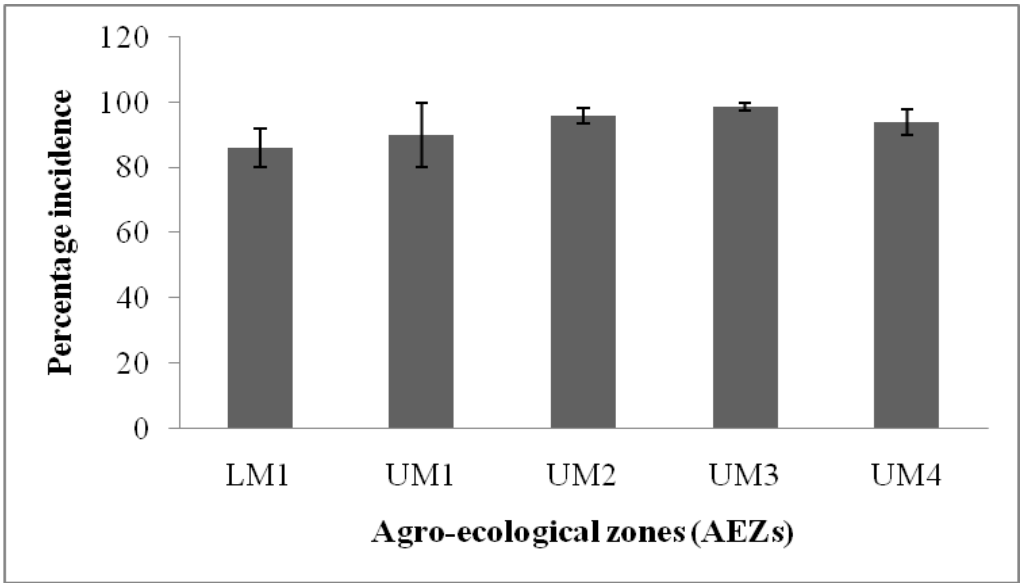

Figure 4.3: Root-knot nematode disease incidence on African nightshades in different agro-ecological zones in Bungoma, Kakamega and Uasin Gishu Counties.

Error bars represents standard error (SE) of the mean.

4.2.2 Root-knot nematode galling index, disease severity and egg-mass index

African nightshade root systems revealed damage by RKN with the development of galls (Figure 4.4 and Plate 1A). Root-knot nematodes galling index (GI) varied significantly ($P < 0.05$) in the sampled areas with an average GI of 2.9 (Figure 4.4). The highest GI that differed significantly ($P < 0.05$) from the other AEZs except UM4 was recorded in UM1 (5.65) followed by UM4 (3.22), UM3 (2.89) and UM2 (2.74) AEZs while the lowest GI that differed significantly ($P < 0.05$) from the other AEZs was recorded in LM1 (1.68) as indicated in Figure 4.4.

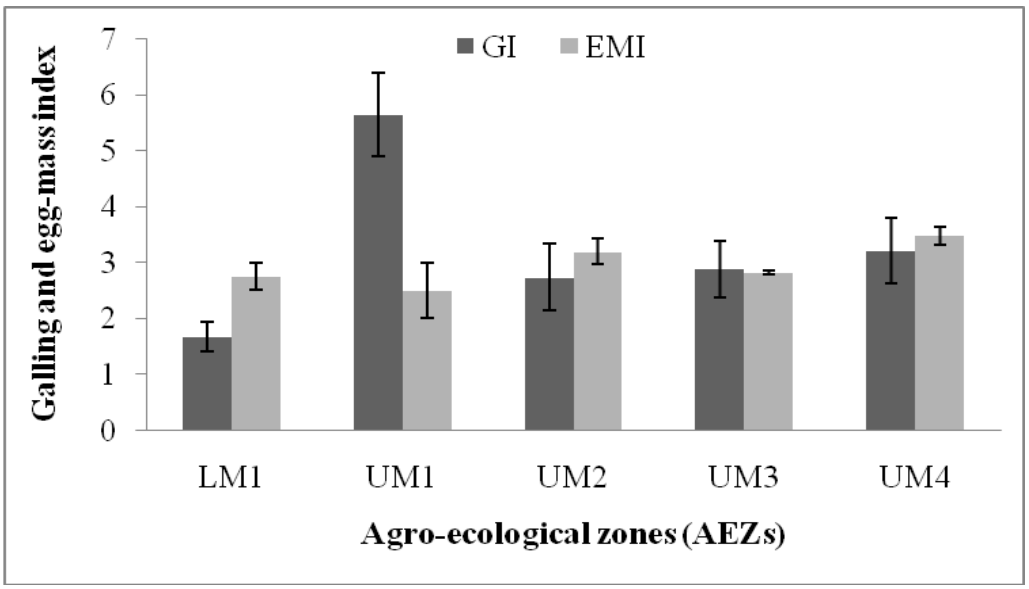

Figure 4.4: Root-knot nematodes galling and egg-mass indices on African nightshades in different agro-ecological zones in Bungoma, Kakamega and Uasin Gishu Counties.

Galling index is based on a scale of 0-10 according to Bridge and Page (1980) while, egg-mass index is based on a scale of 0-5 according to Quesenberry *et al.* (1989).
Error bars represents standard error (SE) of the mean.

Disease severity differed significantly ($P < 0.05$) between the various AEZs. Severe disease intensity was recorded in UM1 (6), followed by UM2, UM3 and UM4 that recorded mild disease intensity (3) while very mild disease severity (2) was recorded in farms in LM1 Agro-ecological zone.

The root system of AFNS revealed RKN egg-masses (Plate 1B) with an average of 2.99 egg-mass index (EMI) that did not differ significantly ($P > 0.05$) between the various AEZs (Figure 4.4). The highest EMI that did not differ significantly ($P > 0.05$) from the other AEZs was recorded in UM4, followed by UM2 (3.2), UM3 (2.83) and LM1 (2.76) while UM1 had the lowest EMI (2.5) that did not differ significantly ($P > 0.05$) from the other AEZs (Figure 4.4).

Plate 1A: African nightshade (*Solanum villosum*) root system heavily galled by root-knot nematodes.

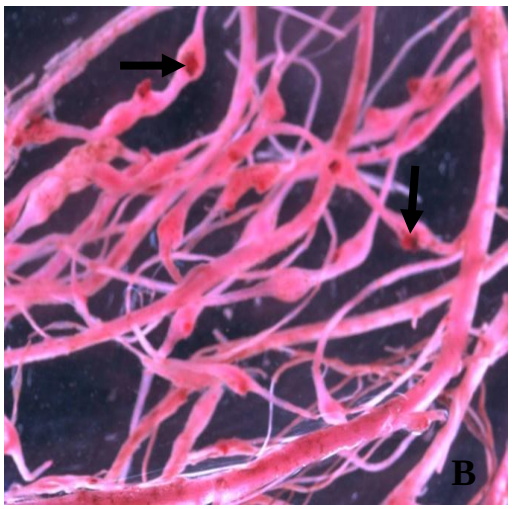

Plate 1B: African nightshade (*S. villosum*) root system showing root-knot nematode galls and egg-masses stained with phloxine B.

4.3 Chemical analysis of soil

Soil textures in the study areas ranged from sandy clay loamy to clay soil (Appendices XI-XIII). Farms in Bungoma County had clay-loam, sandy-clay, clay and sandy-clay-loam texture with the exception of farm 5 and 6 that had sandy-loam texture (Appendix XI). Soil texture in Uasin Gishu and Nandi Counties ranged from loam, sandy-loam, and sandy-clay-loam to clay-loam (Appendix XII). With the exception of farm 1 and 4 that had clay-loam and sandy-clay loam texture, the other farms in Kakamega County had sandy-loam texture (Appendix XIII).

Except for total nitrogen that differed significantly ($P < 0.05$) between the AEZs, there was no significant difference in the mineral content of potassium, phosphorus and soil pH ($P > 0.05$) between the farms in the UM1-UM4 in Uasin Gishu, Nandi and Bungoma Counties, and LM1 in Kakamega County (Figure 4.5). The soil pH ranged from strongly acidic (pH 4.53) through near neutral (pH 6.90) to moderately basic (pH 7.78) as indicated in appendices XI-XIII, with UM1 having more acidic soils (pH = 5.04) while UM2 had slightly acidic (pH = 6.05) soils (Figure 4.5). With the exception of total nitrogen and organic matter that were deficient in the study areas, all the other elements were adequate for growth of AFNS (Appendices XI-XIII). Farms from the study areas had high amounts of phosphorus with soils in UM1-UM4 in Bungoma, Nandi and Uasin Gishu Counties having a higher content ranging from 42.5 – 70.0 % compared to LM1 (38.0 %) in Kakamega County that had the lowest amount of phosphorus (Appendices XI-XIII; Figure 4.5).

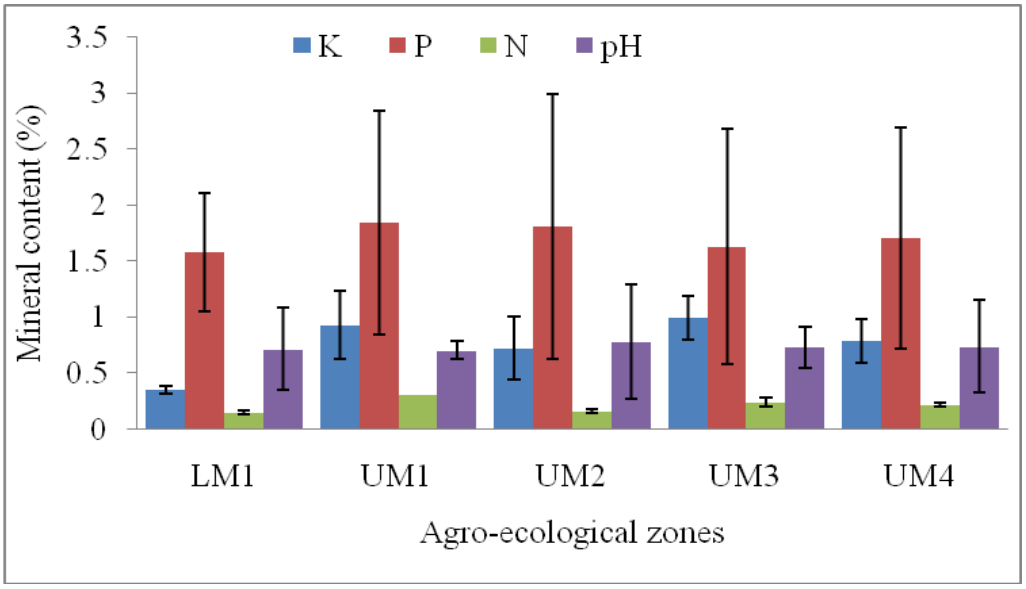

Figure 4.5: Percentage content of potassium, phosphorus and total nitrogen and soil pH in different agro-ecological zones in Bungoma, Kakamega and Uasin Gishu Counties.

Error bars represents standard error (SE) of the mean.

Correlation analysis revealed negative relationship between disease incidence and the amount of phosphorus ($r = -0.0342$, $P > 0.05$) and total nitrogen ($r = -0.1457$, $P > 0.05$) while potassium ($r = 0.4331$, $P < 0.05$) and pH ($r = 0.01637$, $P > 0.05$) had a positive correlation. Except for pH ($r = -0.02867$, $P > 0.05$) that was negatively correlated with galling index, phosphorus ($r = 0.139229$, $P > 0.05$), total nitrogen ($r = 0.18817$, $P > 0.05$) and potassium ($r = 0.1726$, $P > 0.05$) had a positive correlation with GI that did not differ significantly. On the other hand, a positive correlation that did not differ significantly was established between phosphorous ($r = 0.05659$, $P > 0.05$), total nitrogen ($r = 0.015696$, $P > 0.05$) and pH ($r = 0.1292$, $P > 0.05$) while potassium ($r = 0.03903$, $P > 0.05$) had a negative correlation that did not differ significantly.

4.4 Identification of root-knot nematodes on African nightshades

4.4.1 PCR amplification of genomic DNA

The results for PCR amplification of the genomic DNA are presented in Figure 4.6. The band size for the amplified products obtained from the RKN are shown in Figure 4.6. Based on the band size, the RKN species could not be identified thus samples were purified and sent for sequencing.

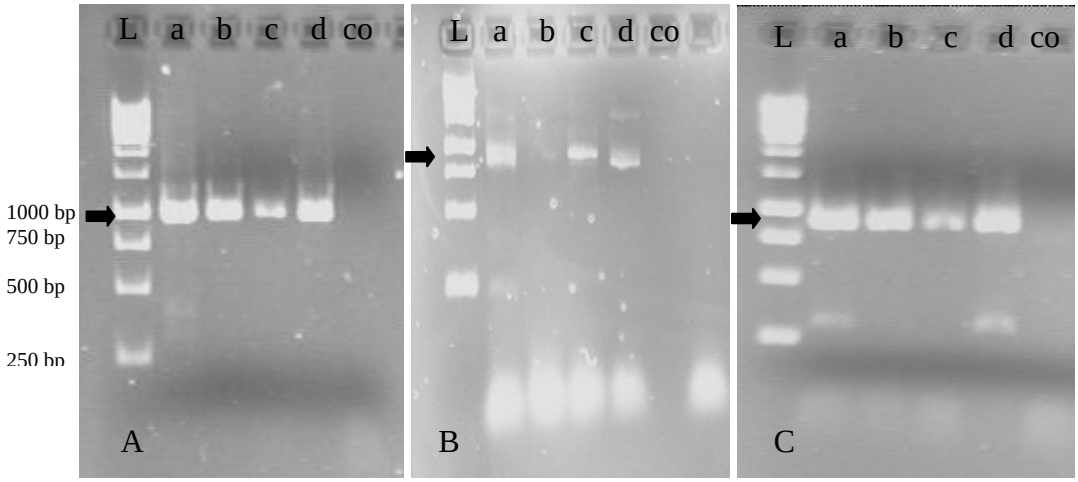

Figure 4.6: Agarose gel amplification of PCR products for 18S rDNA of root-knot nematodes.

Lanes a-d represents isolates KE012, KE014, KE009 and KE010 amplified with primer 988F/1912R targeting 924 bp (A), 1813F/2646R targeting 833 bp (B) and 1096F/1912R targeting 816 bp (C). L - 1 kb ladder (Bioline), co - control.

4.4.2 Phylogenetic studies on root-knot nematodes

Molecular identification was inferred from partially analysed 18S rDNA sequences of 16 root-knot nematode (RKN) isolates (Appendix XIV) with *Nacobbus aberrans*, *Coslenchus cancellatus* and *Subanguina radicicola* as outgroups. The phylogenetic tree is made-up of clades comprising the nematode

isolates and the reference sequences from GenBank (Figure 4.7). The identity of the RKN isolates to GenBank references ranged from 95 % to 99 %.

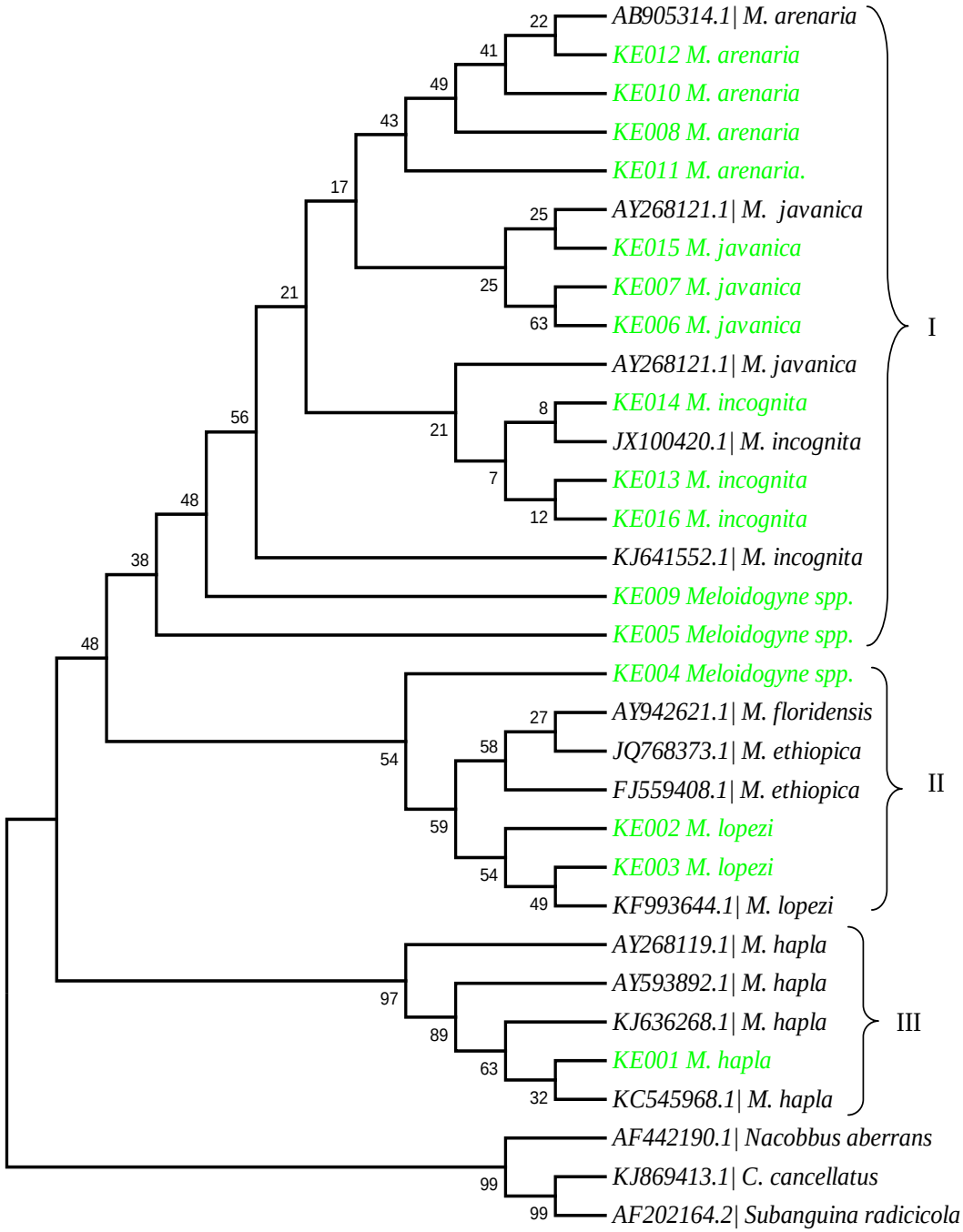

Figure 4.7: Maximum likelihood tree showing the relationship of *Meloidogyne* species isolates based on 18S rDNA gene sequence.

Newly obtained sequences for this study are in green.
Numbers next to branches are bootstrap support values for each clade.

Alignment and phylogenetic analysis of the sequences resulted in three groups separated with varying bootstrap support values in ML analysis (Figure 4.7). The 18S rDNA sequence analysis for nematode isolates from the study clustered together with RKN references retrieved from the GenBank in well supported clades (Figure 4.7). However, the isolates from the surveyed regions did not give identical 18S rDNA to the GenBank references for the same species.

Clade I is made up of mitotic parthenogenetic group comprising of *Meloidogyne incognita*, *M. arenaria* and *M. javanica* (Figure 4.7). In this clade, isolates KE008, KE010, KE011 and KE012 clustered together with *M. arenaria* (AB905314.1) from the GenBank with 47 % bootstrap support. On the other hand, isolates KE006, KE007 and KE015 were sister taxon to *M. javanica* (AY268121.1) in a subgroup that was separated from *M. arenaria* and *M. incognita* subgroups by 16 % and 19 % bootstrap values respectively (Figure 4.7). In another subgroup within clade I, isolates KE013, KE014 and KE016 clustered with *M. incognita* (JX100420.1) from the GenBank. However, the relationship within this subgroup was poorly supported (Figure 4.7). Although isolates KE005 and KE009 were positioned as sister taxon with *M. incognita* (KJ6415552.1) from GenBank in clade I, their identity was not resolved and thus they were identified as *Meloidogyne* species (Figure 4.7).

Isolates KE002, KE003 and KE004 were sister taxon to *M. lopezi* (KF993644.1), *M. ethiopica* (FJ559408.1 and JQ768373.1) and *M. floridensis* (AY942621.1) from the GenBank in clade II supported by 48 % bootstrap (Figure 4.7). In this subgroup, isolates KE002 and KE003 clustered with *M. lopezi* (KF993644.1) supported by 51-54 % bootstrap value, while isolate

KE004 positioned as siter taxon to other isolated and *M. lopezi* (KF993644.1), *M. ethiopica* (FJ559408.1 and JQ768373.1) and *M. floridensis* (AY942621.1) in clade II, was identified as *Meloidogyne* spp. as indicated in Figure 4.7.

Isolate KE001 was sister taxon to amphimictic *M. hapla* (KJ636268.1; KC545968.1; AY593892.1 and AY268119.1) in clade III supported with 96 % bootstrap value (Figure 4.7). Within this clade, *M. hapla* (Isolate KE001) was closely related to *M. hapla* (KJ636268.1) from the GenBank although the relationship was poorly supported (Figure 4.7). All the isolates were distantly related to *C. cancellatus* and *S. radicicola* outgroups (Figure 4.7).

The different species of *Meloidogyne* associated with AFNS are presented in Table 4.6. Of all the RKN, *Meloidogyne arenaria* constituted 25.0 % occuring in Upper midland zone (UM1) and UM2, while *M. incognita* identified in UM3 and UM4, *M. javanica* identified in UM1 and UM4 and *Meloidogyne* spp. in UM2 and UM3 constituted of 18.75 % respectively (Table 4.6). On the other hand, *M. lopezi* that was identified from LM1 and UM4 recorded 12.5 % occurrence, while *M. hapla* identified from UM4 had the lowest percentage occurrence (6.25 %) compared with the other species (Table 4.6).

Table 4.6: Distribution of root-knot nematodes in the surveyed region

Root-knot nematode species	Agro-ecological zone	% Distribution
Meloidogyne arenaria	UM2, UM3	25.0
M. hapla	UM4	6.25
M. incognita	UM3, UM4	18.75
M. javanica	UM1, UM4	18.75
M. lopezi	LM1, UM4	12.5
Meloidogyne spp.	UM2,UM3	18.75

4.4.3 Nucleotide frequencies (%)

The nucleotide frequencies for 16 nematode isolate sequences were 26.8 % (A), 26.4 % (T/U), 20.7 % (C), and 26.1 % (G) as shown in Table 4.7. The T+A nucleotides had 53.2 % compositions while the G+C was 46.8 % (Table 4.7).

Table 4.7: Nucleotide frequencies

Isolates	Thymine	Cytosine	Adenine	Guanine	Total
KE001	25.7	21.4	26.8	26.2	1694.0
KE002	26.7	20.8	26.4	26.0	1675.0
KE003	26.7	20.4	26.7	26.1	1351.0
KE004	26.2	20.7	26.8	26.3	1437.0
KE005	26.8	20.2	27.1	25.9	1380.0
KE006	26.5	20.4	27.2	25.9	1376.0
KE007	26.6	20.9	26.4	26.1	1691.0
KE008	25.7	20.6	27.6	26.1	898.0
KE009	26.8	20.8	26.6	25.8	1700.0
KE010	27.5	20.9	26.5	25.1	1843.0
KE011	26.6	20.4	26.6	26.4	1678.0
KE012	25.9	21.1	26.9	26.1	1513.0
KE013	26.1	21.3	26.4	26.1	1649.0
KE014	26.4	20.7	26.7	26.2	1707.0
KE015	26.0	21.0	26.7	26.4	863.0
KE016	26.1	20.1	27.4	26.4	1748.0
Average	26.4	20.7	26.8	26.1	1512.7

4.4.4 Tajima's neutrality test

The results for Tajima analysis for 16 nucleotide sequences are shown in Table 4.8. There were a total of 829 positions in the final dataset. The nucleotide diversity observed from the alignment of the 16 sequences was 0.027925 and a Tajima test statistic of -1.922706 (Table 4.8).

Table 4.8: Tajima's neutrality test for 16 sequences

M	*S*	p_s	Θ	π	*D*
16	138	0.166466	0.050167	0.027925	-1.922706

M = number of sequences, *n* = total number of sites, *S* = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and *D* is the Tajima test statistic.

4.4.5 Maximum composite likelihood estimate of the pattern of nucleotide substitution

The results for each entry showing the probability of substitution from one base (row) to another base (column) instantaneously with only the entries within a row being compared are shown in Table 4.9. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The transition/transversion rate ratios are k1 = 3.141 (purines) and k2 = 3.998 (pyrimidines). The overall transition/transversion bias is R = 1.755, where R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]. The analysis involved 32 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 628 positions in the final dataset.

Table 4.9: Nucleotide substitution

	A	**T**	**C**	**G**
A	-	*4.81*	*3.64*	**14.77**
T	*4.89*	-	**14.56**	*4.7*
C	*4.89*	**19.24**	-	*4.7*
G	**15.35**	*4.81*	*3.64*	-

Transitional substitutions are shown in bold while transversional substitutions

4.5 Response of African nightshades to root-knot nematodes in the greenhouse experiment at Kenyatta University

4.5.1 Plant growth parameters

Plants grown on nematode inoculated soils had shorter shoots that did not differ significantly ($P < 0.05$) from those grown on non-inoculated soils (Figure 4.8). Compared to the positive control (-19.8 %), the highest reduction on shoot height (SH) was observed in *S. nigrum* from Simlaw seeds (-25.5 %).

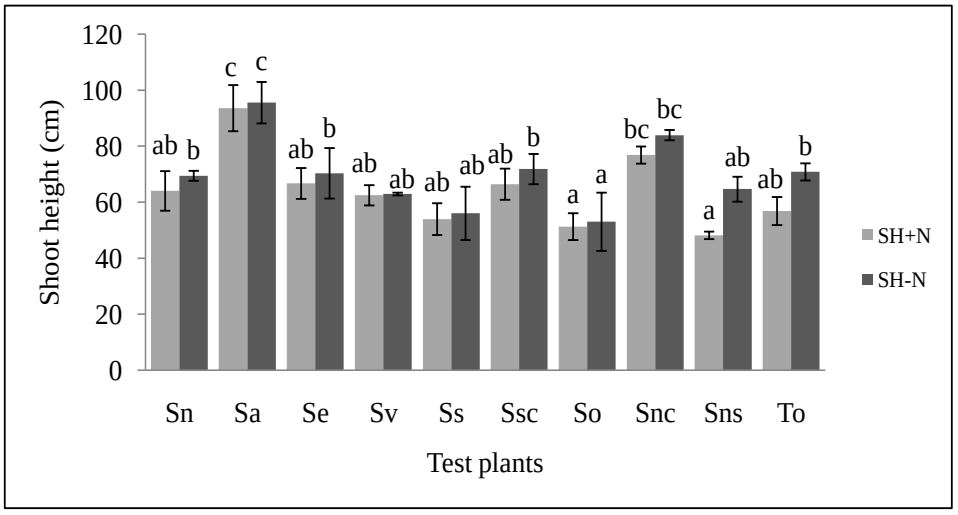

Figure 4.8: Mean shoot height (cm) of African nightshades and tomato 60 days after inoculation with root-knot nematodes in the greenhouse.

Data is a mean of four replications. Error bars represents standard error (SE) of the mean. Bars followed by similar letter (s) are not statistically different ($P > 0.05$) according to LSD test.
SH+N Nematode inoculated, SH-N Non-inoculated. Sn - *Solanum nigrum* line IP03, Sa - *S. americanum* line RC01, Se - *S. eldoretiunum* line MW05, Sv - *S. villosum* line BG03, Ss - *S. sarrarachoides* line MW13, Ssc - *S. scabrum* line RV1, So - *S. opacum* landrace from Kisii, Snc - *S. nigrum* landrace from Kakamega, Sns - *S. nigrum* (Simlaw) and To - Money-maker tomato.

Lower SH reduction compared to the positive control was recorded on S. *nigrum* landrace from Kakamega (-8.5 %), *S. nigrum* line IP03 (-7.8 %), *S.*

eldoretiunum line MW05 (-5.2 %), *S. scabrum* line RV1 (-7.5 %), *S. sarrarachoides* line MW13 (-3.8 %), *S. opacum* from Kisii (-3.2 %), *S. americanum* line RC01 (-2.1 %) and *S. villosum* line BG03 (-0.064 %) as shown in Figure 4.8.

Significantly ($P < 0.05$) higher reduction in dry shoot weight was recorded on *S. villosum* line BG03, followed by *S. nigrum* line IP03, *S. nigrum* (Simlaw) and *S. sarrarachoides* line MW13 relative to tomato (Table 4.10). All the other AFNS had lower reductions in dry shoot weight that did not differ significantly ($P > 0.05$) with the positive control (Table 4.10).

Table 4.10: Mean[#] dry shoot weight and fresh root weight (g) of African nightshades and tomato in the greenhouse at Kenyatta University

AFNS	Plant parameters	Inoculated	Non-inoculated	% change	*t*-test (means)
Solanum nigrum (IP03)	Dry shoot weight	5.83	12.4	-48.5	S
	Fresh root weight	26.35	22.13	+20.0	NS
S. americanum (RC01)	Dry shoot weight	11.38	13.16	-13.7	NS
	Fresh root weight	25.83	25.38	+2.3	NS
S. eldoretiunum (MW05)	Dry shoot weight	10.34	12.08	-13.5	NS
	Fresh root weight	42.00	41.0	+3.3	NS
S. villosum (BG03)	Dry shoot weight	6.91	14.75	-52.3	S
	Fresh root weight	26.54	22.3	+19.2	NS
S. sarrarachoides (MW13)	Dry shoot weight	11.68	17.6	-34.4	NS
	Fresh root weight	27.38	21.28	+34.7	NS
S. scabrum (RV1)	Dry shoot weight	12.5	13.93	-10.3	NS
	Fresh root weight	44.78	42.7	+5.0	NS
S. opacum	Dry shoot weight	15.95	16.98	-5.9	NS
	Fresh root weight	20.6	16.48	+30.6	NS
S. nigrum (Kakamega)	Dry shoot weight	18.93	20.98	-9.1	NS
	Fresh root weight	38.00	36.18	+5.3	NS
S. nigrum (Simlaw)	Dry shoot weight	8.48	15.6	-45.2	S*
	Fresh root weight	21.9	16.98	+30.9	NS
S. lycopersicum (Tomato)	Dry shoot weight	10.2	15.53	-33.5	S
	Fresh root weight	23.9	16.53	+45.5	S*

[#] Data is a mean of four replications.
NS- Not significant ($P > 0.05$), S- significant ($P < 0.05$), S*- highly significant ($P \leq 0.001$).

Except for the positive control that had significant ($P < 0.05$) increase in fresh root, all the other AFNS recorded an increase in fresh root weight that did not differ significantly ($P > 0.05$). The highest reduction in fresh root weight was recorded on *S. sarrarachoides* line MW13 (34.7 %), followed by *S. nigrum* from Simlaw (30.9 %) and *S. opacum* (30.6 %) as indicated in Table 4.10. Lower increase in fresh root weight was recorded in *S. scabrum* line RV1 (5.9 %), followed by *S. nigrum* landrace from Kakamega (5.3 %) and *S. americanum* line RC01 (2.3 %) that had the lowest increase in fresh root weight (Table 4.10).

4.5.2 Root-knot nematode disease parameters

Root-knot nematode J2 populations differed significantly ($P < 0.05$) between the AFNS (Table 4.11). All the AFNS except *S. villosum* line BG 03 and *S. sarrarachoides* line MW13 had lower J2 populations compared with the positive control. Significantly ($P < 0.05$) lower J2 populations compared with the positive control were recorded in *S. nigrum* landrace from Kakamega, followed by *S. opacum*, *S. scabrum* line RV1, *S. americanum* line RC01 and *S. eldoretiunum* line MW05, while *S. nigrum* line IP03 and *S. nigrum* (Simlaw) had lower J2 populations that did not differ significantly ($P > 0.05$) with the positive control (Table 4.11).

Although the galling index (GI) did not differ significantly ($P > 0.05$) between the AFNS, significantly ($P < 0.05$) lower GI compared with the positive control were recorded on all the AFNS except *S. americanum* line RC01, *S. nigrum* line IP03, *S. opacum* and *S. villosum* line BG03 (Table 4.11).

Root-knot nematode reproduced on all the AFNS in the greenhouse condition (Table 4.11). The nematode reproduction varied significantly ($P <$

0.05) between the AFNS (Table 4.11). The highest reproduction factor (Rf) that did not differ significantly (P > 0.05) from the control was recorded on *S. sarrarachoides* line MW13 (Table 4.11). All the other AFNS had significantly (P < 0.05) lower RKN reproduction compared to the positive control (Table 4.11).

The host status of AFNS to RKN in the greenhouse varied from resistant to tolerant compared with the susceptible control (Table 4.11). With the exception of *S. sarrarachoides* line MW13 and *S. nigrum* from Simlaw Seed Company that were tolerant, all the other AFNS were resistant to RKN (Table 4.11).

Table 4.11: Mean[#] J2 population, nematode reproduction factor (Rf), galling index (GI) and host status in the greenhouse at Kenyatta University

African nightshades	J2 population[w]	Galling index (GI[y])	Reproduction factor (Rf[x])	Host status[z]
Solanum americanum (RC01)	2.3 bc	0.64 abc	0.20 d	R
S. nigrum (IP03)	2.69 ab	0.66 abc	0.98 bc	R
S. eldoretiunum (MW05)	2.26 bc	0.62 bc	0.73 cd	R
S. nigrum (Kakamega)	1.53 d	0.45 bc	0.28 d	R
S. opacum	2.09 c	0.71 ab	0.68 cd	R
S. sarrarachoides (MW13)	2.93 a	0.62 bc	2.03 ab	T
S. scabrum (RV1)	2.25 bc	0.48 bc	0.63cd	R
S. nigrum (Simlaw)	2.75 ab	0.55 bc	1.05 bc	T
S. villosum (BG03)	2.90 a	0.7 abc	0.35 cd	R
Tomato	2.85 a	0.9 a	5.37 a	S
P-value	0.0001	0.0552	0.0001	
LSD	0.5385	0.2554	0.1118	

[#] Data for J2 and galling index are mean ($Log_{10}(x+1)$) of four replications.
Means within the same column followed by similar letter (s) are not statistically different (P > 0.05) according to LSD test.
[w] Nematode population per 100 cc soil.
[x] Rf (nematode reproduction factor) = ratio of final nematode population (Pf) to the initial nematode population (Pi).
[y] Galling index (GI) on a scale of 0-10 according to Bridge and Page (1980).
[z] Host status based on Rf according to Zhang and Schmitt (1994) where; Immune (I) if Rf = 0; resistant (R) if 1 > Rf > 0; tolerant (T) if 5 ≥ Rf > 1 and susceptible (S) if Rf > 5.

4.5.3 Response of African nightshades to root-knot nematodes in the field screening experiment at Kenyatta University

4.5.3.1 Plant growth parameters

Except for tomato that had significantly ($P < 0.05$) shorter shoots, all the AFNS grown on nematode inoculated soils recorded shorter shoots that did not differ significantly ($P > 0.05$) from those grown on non-inoculated soils (Figure 4.9).

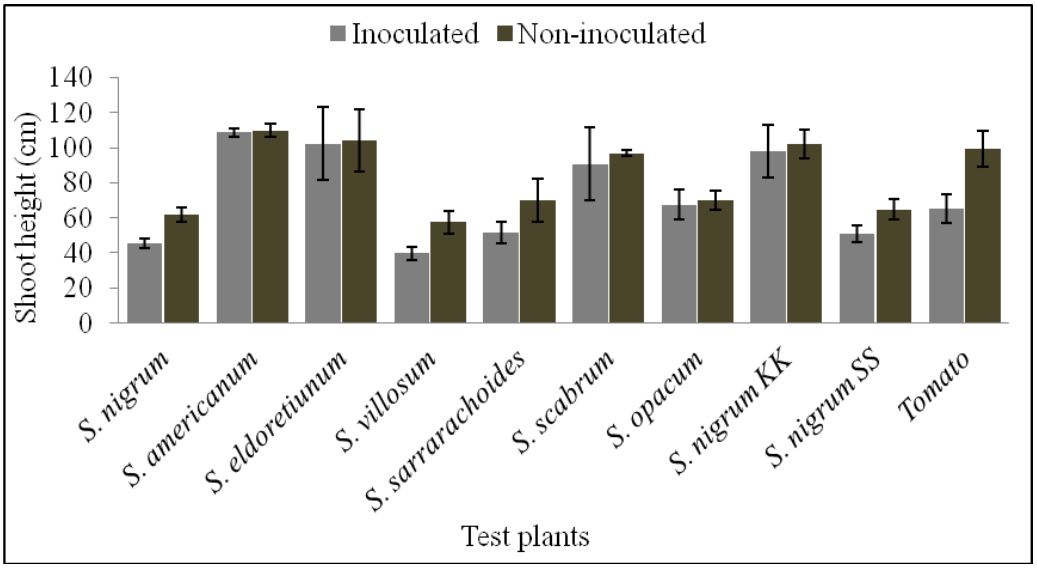

Figure 4.9: Effect of root-knot nematodes on shoot height of African nightshades and tomato in the field at Kenyatta University.

Data are means ± SE of three replications/African nightshade.
Shoot height reduction expressed as a percentage of control – inoculated divided by control. KK – Kakamega, SS – Simlaw Seed Company.

The highest reduction in shoot heigth was recorded on *S. villosum* line BG03 (-30.78 %), followed by *S. nigrum* line IP03 (-26.58 %), *S. sarrarachoides* line MW13 (-26.43 %), *S. nigrum* from Simlaw Seed Company (-21.26 %), *S. scabrum* line RV1 (-6.32 %), *S. opacum* (-3.85 %), *S.*

eldoretiunum line MW05 (-1.73 %) and *S. americanum* line RC01 (-1.03 %), while *S. nigrum* landrace from Kakamega (-6.32 %) had the lowest reduction compared to the positive control (-34.14 %) as shown in Figure 4.9.

Although the dry shoot weight of AFNS grown on soils infested with RKN were lower than those grown on non-infested soils, there was no significant difference ($P > 0.05$) established except for *S. americanum* line RC01 and *S. eldoretiunum* line MW05 that differed significantly ($P < 0.05$) as indicated in Table 4.12. Compared to the positive control, the highest reduction in dry shoot weight was recorded on *S. americanum* line RC01, followed by *S. nigrum* landrace from Kakamega and *S. villosum* line BG03, while all the other AFNS had lower reduction in dry shoot weight that did not differ significantly except for *S. eldoretiunum* line MW05 (Table 4.12).

The fresh root weights of AFNS grown on nematode infested soils were significantly ($P < 0.05$) heavier compared to those grown on non-infested soils except for *S. americanum* line RC01, *S. eldoretiunum* line MW05, *S. scabrum* line RV1 and *S. opacum* that were not significantly different (Table 4.12). Compared to the positive control, the increase in fresh root weight was lower in all the AFNS. *Solanum sarrarachoides* line MW13 had the highest increase in fresh root weight, followed by *S. villosum* line BG03, while *S. americanum* line RC01, *S. eldoretiunum* line MW05, *S. nigrum* from Kakamega and *S. opacum* recorded lower increase in fresh root weight compared with the positive control (Table 4.12).

Table 4.12: Mean[#] dry shoot weight and fresh root weight of African nightshades (AFNS) in the field at Kenyatta University

AFNS	Plant parameters in grams	Infested soil	Non-infested[x]	% change	*t*-test (means)
Solanum nigrum (IP03)	Dry shoot weight	28.02	38.3	-26.84	NS
	Fresh root weight	15.17	11.67	+29.9	S
S. americanum (RC01)	Dry shoot weight	17.84	56.8	-68.59	S
	Fresh root weight	12.25	11.58	+5.8	NS
S. eldoretiunum (MW05)	Dry shoot weight	11.55	25.89	-55.39	S
	Fresh root weight	12.10	12.02	+0.7	NS
S. villosum (BG03)	Dry shoot weight	10.4	25.6	-59.38	NS
	Fresh root weight	16.13	11.1	+45.3	S
S. sarrarachoides (MW13)	Dry shoot weight	26.3	33.8	-22.19	NS
	Fresh root weight	14.4	9.7	+48.5	S
S. scabrum (RV1)	Dry shoot weight	28.6	36.3	-21.21	NS
	Fresh root weight	19.5	18.7	+4.3	NS
S. opacum	Dry shoot weight	24.33	30.9	-21.26	NS
	Fresh root weight	10.26	9.52	+7.8	NS
S. nigrum (Kakamega)	Dry shoot weight	12.04	35.9	-66.46	NS
	Fresh root weight	13.53	12.15	+11.4	S
S. nigrum (Simlaw)	Dry shoot weight	17.54	21.96	-20.13	NS
	Fresh root weight	17.8	12.09	+5.7	S
S. lycopersicum (Tomato)	Dry shoot weight	10.87	25.6	-57.54	NS
	Fresh root weight	14.9	9.78	+52.4	S

[#] Data are mean ± SE of three replications/African nightshade.
NS- Not significant ($P > 0.05$), S- significant ($P < 0.05$), S*- highly significant ($P \leq 0.001$) according to two-sample t-test.
[x] Plots were treated with 200 ml ha^{-1} (0.078 ml $plot^{-1}$) of Real Trichoderma®

4.5.3.2 Root-knot nematode disease parameters in the field at Kenyatta University

Nematode populations differed significantly ($P < 0.05$) between the various AFNS (Table 4.13). The highest J2 populations that did not differ significantly ($P > 0.05$) from the positive control (tomato) were found on *S. nigrum* line IP03 and *S. sarrarachoides* line MW13, while all the other AFNS had lower J2 populations that differed significantly ($P < 0.05$) from the positive control (Table 4.13).

Table 4.13: Mean[#] J2 population, reproduction factor, galling index and host status of African nightshades (AFNS) in field at Kenyatta University

AFNS	J2 population	Galling index (GI)[x]	Reproduction factor (Rf)[w]	Host status[y]
Solanum americanum (RC01)	2.35[c]	0.07[b]	1.13[bc]	T
S. nigrum (IP03)	3.01[ab]	0.73[a]	3.103[ab]	T
S. eldoretiunum (MW05)	2.0[d]	0.28[b]	0.183[c]	R
S. nigrum (Kakamega)	2.0[d]	0.18[b]	0.262[c]	R
S. opacum	2.43[c]	0.29[b]	0.862[bc]	R
S. sarrarachoides (MW13)	2.98[ab]	0.79[a]	3.382[a]	T
S. scabrum (RV1)	1.90[d]	0.18[b]	0.276[c]	R
S. nigrum (Simlaw)	2.88[b]	0.81[a]	2.338[ab]	T
S. villosum (BG03)	2.91[b]	0.76[a]	2.043[ab]	T
Tomato	3.21[a]	0.87[a]	6.702[a]	S
P-value	0.0001	0.0001	0.0003	
LSD	0.2577	0.2876	0.5281	

[#]Data for J2 and galling index are mean ($Log_{10}(x+1)$) of three replications/African nightshade. Means on the same column followed by similar letter(s) are not statistically different ($P > 0.05$) according to the LSD test.
[w] Rf (nematode reproduction factor) = ratio of final nematode population (Pf) to the initial nematode population (Pi).
[x] Galling index based on a scale of 0-10 (Bridge and Page, 1980).
[y] Host status based on Rf according to Zhang and Schmitt (1994) where; Immune (I) if $Rf = 0$; resistant (R) if $1 > Rf > 0$; tolerant (T) if $5 \geq Rf > 1$ and susceptible (S) if $Rf > 5$.

Root-knot nematode galling index (GI) and reproduction (Rf) differed significantly ($P > 0.05$) between the various AFNS and the positive control (Table 4.13). Except for *S. nigrum* line IP03, *S. nigrum* from Simlaw Seed Company, *S. sarrarachoides* line MW13 and *S. villosum* line BG03 that had lower GI did not differ significantly ($P > 0.05$) from the positive control, all the AFNS had significantly ($P < 0.05$) lower GI compared with the control with the lowest GI being recorded on *S. americanum* line RC01 (Table 4.13). Similarly, reproduction factor differed significantly ($P < 0.05$) with all the AFNS except *S. nigrum* line IP03, *S. nigrum* from Simlaw Seed Company, *S. sarrarachoides*

line MW13 and *S. villosum* line BG03 recording lower Rf that did not differ significantly (P > 0.05) from the positive control (Table 4.13).

The response of the various AFNS to RKN infection varied from resistant to tolerant in the field test at Kenyatta University (Table 4.13). Whereas *S. eldoretiunum* line MW05, *S. nigrum* landrace from Kakamega, *S. opacum* and *S. scabrum* line RV1 were resistant to RKN, the response of *S. americanum* line RC01, *S. nigrum* line IP03, *S. nigrum* from Simlaw Seed Company, *S. sarrarachoides* line MW13 and *S. villosum* line BG03 were tolerant compared with the susceptible positive control (Table 4.13).

4.5.4 Response of African nightshades to RKN in the field screening experiment at Chepterwai

4.5.4.1 Plant growth parameters

Shoots of AFNS grown on RKN infested soil were shorter than those grown on non-infested soil with no significant difference established (Figure 4.10). The highest reduction in shoot height was recorded on *S. sarrarachoides* line MW13 (-51.6 %), followed by *S. villosum* line BG03 (-39.0 %) and *S. nigrum* from Simlaw (-36.1 %) compared with positive control (-33.0 %) as shown in Figure 4.10. *Solanum nigrum* line IP03 (-15.4 %), *S. americanum* line RC01 (-14.0 %), *S. scabrum* line RV1 (-9.2 %), *S. opacum* (-7.2 %), *S. nigrum* landrace from Kakamega (-6.2 %) and *S. eldoretiunum* line MW05 (-5.0 %) had lower reduction in shoot height compared to the positive control (Figure 4.10).

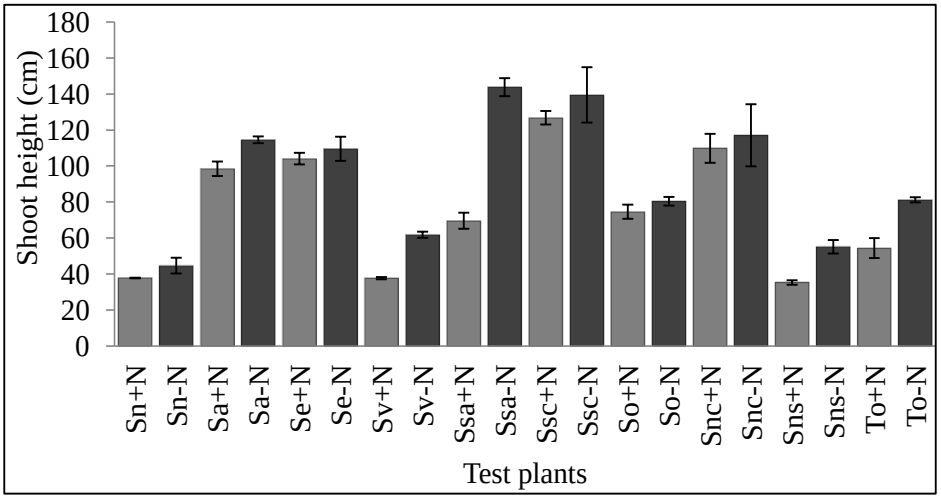

Figure 4.10: Effect of root-knot nematodes on shoot height of African nightshades in the field test at Chepterwai.

Data are mean ± SE of three replications/African nightshade. +N = Nematode inoculated; -N = Non-inoculated. Sn - *Solanum nigrum* line IP03, Sa - *S. americanum* line RC01, Se - *S. eldoretiunum* line MW05, Sv - *S. villosum* line BG03, Ssa - *S. sarrarachoides* line MW13, Ssc - *S. scabrum* line RV1, So - *S. opacum*, Snc - *S. nigrum* landrace from Kakamega, Sns - *S. nigrum* from Simlaw Seed Company and To - Money-maker tomato (Positive control).

Dry shoot weight of AFNS grown on RKN infested soils was lower compared to those grown on non-infested soils although there was no significant difference established (Table 4.14). Except for the positive control that had a higher significant ($P < 0.05$) reduction on dry shoot weight (-34.8 %), all the AFNS had dry shoot weight reductions that did not differ significantly ($P > 0.05$) with reduction in *S. sarrarachoides* line MW13 (-29.2 %), *S. villosum* line BG03 (-27.7 %) and *S. nigrum* from Simlaw Seed Company (19.2 %) being the highest, while that of *S. americanum* line RC01 (-1.4 %) was the lowest compared with the positive control (Table 4.14).

Table 4.14: Mean[#] dry shoot weight and fresh root weight of African nightshades (AFNS) in the field test at Chepterwai

AFNS	Plant parameters	With RKN	Without RKN[x]	% change	*t*-test (means)
Solanum nigrum (IP03)	Dry shoot weight	6.12	9.10	-3.3	NS
	Fresh root weight	8.05	5.13	+56.9	S
S. americanum (RC01)	Dry shoot weight	36.03	37.02	-2.7	NS
	Fresh root weight	13.03	12.93	+0.8	NS
S. eldoretiunum (MW05)	Dry shoot weight	33.53	34.72	-3.43	NS
	Fresh root weight	14.88	13.94	+6.74	NS
S. villosum (BG03)	Dry shoot weight	13.16	18.19	-27.7	NS
	Fresh root weight	6.86	4.27	+60.7	S
S. sarrarachoides (MW13)	Dry shoot weight	12.75	18.08	-29.2	NS
	Fresh root weight	6.37	4.78	+33.3	NS
S. scabrum (RV1)	Dry shoot weight	308.3	356.49	-13.5	NS
	Fresh root weight	25.93	26.78	+3.2	NS
S. opacum	Dry shoot weight	23.96	25.34	-5.5	NS
	Fresh root weight	7.5	7.16	+4.8	NS
S. nigrum (Kakamega landrace)	Dry shoot weight	29.81	30.23	-1.4	NS
	Fresh root weight	11.85	11.16	+6.2	NS
S. nigrum (Simlaw)	Dry shoot weight	11.77	14.56	-19.2	NS
	Fresh root weight	7.21	3.77	+91.3	S
S. lycopersicum (Tomato)	Dry shoot weight	14.15	21.71	-34.8	S
	Fresh root weight	7.81	4.2	+86.0	S

[#] Data are mean of three replications/African nightshade.
NS- Not significant ($P > 0.05$), *- significant ($P < 0.05$), S*- highly significant ($P \leq 0.001$) with two-sample t-test.
[x] Plots were treated with 200 ml ha^{-1} (0.078 ml $plot^{-1}$) of Real Trichoderma®

Fresh root weight of AFNS grown on soils infested with RKN did not differ significantly ($P > 0.05$) from those grown on soils non-infested, except for *S. nigrum* line IP03, *S. villosum* line BG03, *S. nigrum* from Simlaw Seed Company and the positive control that differed ($P < 0.05$) significantly (Table 4.14). The AFNS grown on infested soils had heavier roots compared to those grown on soils non-infested soil (Table 4.14). Except for *S. nigrum* from Simlaw Seed Company (91.3 %), the positive control (86.0 %), *S. villosum* line BG03 (60.7 %) and *S. nigrum* line IP03 (56.9 %) that had significant ($P < 0.05$)

increase in fresh root weight, all the other AFNS had fresh root weight increase that were not significant. The lowest increase in fresh root weight was recorded in *S. americanum* line RC01 compared to the positive control (Table 4.14).

4.5.4.2 Root-knot nematode disease parameters in field test at Chepterwai

The J2 population differed significantly ($P < 0.05$) with all the AFNS except *S. sarrarachoides* line MW13 recording significantly ($P < 0.05$) lower J2 populations compared to the positive control, the J2 population for all the other AFNS was higher (Table 4.15).

The GI differed significantly ($P < 0.05$) between the treatments (Table 4.15). All the AFNS recorded significantly ($P < 0.05$) lower GI compared with the positive control. The GI was higher in *S. nigrum* Simlaw Seed Company, *S. sarrarachoides* line MW13, *S. nigrum* line IP03 and *S. villosum* line BG03, while significantly ($P < 0.05$) lower galling indices were recorded in *S. opacum*, *S. eldoretiunum* line MW05, *S. nigrum* landrace from Kakamega, *S. americanum* line RC01 and *S. scabrum* line RV1 compared to the positive control (Table 4.15).

On the other hand, RKN reproduction factor did not differ significantly ($P > 0.05$) between the treatments in the field test at Chepterwai. All the AFNS except *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 had significantly ($P < 0.05$) lower reproduction factor compared to the positive control (Table 4.15).

Table 4.15: Mean[#] J2 population, reproduction factor (Rf), galling index (GI) and host status of African nightshades in the field at Chepterwai

AFNS	J2 population	GI[x]	Rf[w]	Host status[y]
Solanum americanum (RC01)	2.26 d	0.22 cd	1.1 b	T
S. nigrum (IP03)	2.37 cd	0.63 b	1.27 b	T
S. eldoretiunum (MW05)	2.22 d	0.28 cd	1.00 b	R
S. nigrum (Kakamega)	2.26 d	0.21 cd	1.55 b	T
S. opacum	2.91 d	0.34 c	1.96 b	T
S. sarrarachoides (MW13)	2.82 ab	0.71 b	3.67 b	T
S. scabrum (RV)	2.21 d	0.16 d	0.97 b	R
S. nigrum (Simlaw)	2.86 ab	0.73 b	6.19 ab	S
S. villosum (BG03)	2.65 bc	0.66 b	5.03 ab	S
Tomato	3.01 a	0.86 a	12.03 a	S
P-value	0.0001	0.0001	0.0772	
LSD	0.3403	0.1284	0.7100	

[#] Data for J2 and galling index are mean ($Log_{10}(x+1)$) of three replications/ African nightshade. Means within the same column followed by similar letter (s) are not statistically different ($P > 0.05$) according to the LSD test.
[w] Rf (nematode reproduction factor) = ratio of final nematode population (Pf) to the initial nematode population (Pi).
[x] Galling index (GI) scale of 0-10 according to Bridge and Page (1980).
[y] Host status based on Rf according to Zhang and Schmitt (1994) where; Immune (I) if $Rf = 0$; resistant (R) if $1 > Rf > 0$; tolerant (T) if $5 \geq Rf > 1$ and susceptible (S) if $Rf > 5$.

The host status of AFNS to RKN varied from resistant to susceptible in the field test at Chepterwai (Table 4.15). Exception for *S. eldoretiunum* line MW05 and *S. scabrum* line RV1 that were resistant to RKN, *S. americanum* line RC01, *S. nigrum* line IP03, *S. nigrum* landrace from Kakamega, *S. opacum* and *S. sarrarachoides* line MW13 were tolerant while, *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 were susceptible similar to the positive control (Table 4.15).

4.6 Efficacy of solarizing soils amended with and without selected organic materials on root-knot nematode on African nightshades under field conditions at Chepterwai

4.6.1 Root-knot nematode damage on African nightshade

The galling index (GI) and egg-mass index (EMI) differed significantly ($P < 0.05$) between the treatments (Table 4.16). *Solanum villosum* grown on solarized soils amended with Cm, Td and Pm and unamended control had lower GI and EMI compared with those grown on non-solarized soils (Table 4.16).

Table 4.16: Effect of solarization on galling index and egg-mass index on *S. villosum* in the field test at Chepterwai

Amendments	Treatments	Galling index[y]	Egg-mass index[z]
Cattle manure (Cm)	Solarized	0.7c[x]	2.0cd
	Non-solarized	2.9ab	3.3ab
Tithonia compost (Td)	Solarized	1.8bc	1.3d
	Non-solarized	2.5b	2.3bc
Pymarc (Pm)	Solarized	1.3bc	2.0cd
	Non-solarized	1.4bc	2.3bc
Control	Solarized	2.2bc	4.7a
	Non-solarized	5.0a	5.0a
P-value		0.0175	0.0001
LSD		0.2921	0.1381

[x] Data are means of three replications. Means on the same column followed by similar letter(s) are not significantly different at $P \geq 0.05$ according to LSD test.
[y] Galling index scale of 0-10 where 0 = no galls, 5 = 50% of the roots infected and 10 = entire root system galled and plants usually dead (Bridge and Page, 1980).
[z] Egg-mass index on a 0-5 scale where; 0 = no egg-masses; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100 and 5 = > 100 egg-masses per root system (Quesenberry *et al*., 1989).

Although solarized soils amended with Td and Pm recorded lower GI that did not differ significantly ($P > 0.05$) from non-solarized soils, the EMI for solarized soils amended with Td was significantly lower ($P < 0.05$) compared to that of non-solarized soils (Table 4.16). Solarization and amendment effect on GI and EMI was significant ($P < 0.05$) although the interaction effect of solarization × amendment on both GI and EMI was not significant (Appendices XV and XVI).

4.6.2 Root-knot nematode population

The nematode populations did not differ significantly ($P > 0.05$) among the plots before solarization (Table 4.17). However, at the end of solarization, the RKN population differed significantly ($P < 0.05$) between the treatments. The J2 population was suppressed on solarized amended soils compared with non-solarized soils (Table 4.17). Except for solarized soils amended with Cm that significantly suppressed RKN population compared to non-solarized soil, all the other treatments did not differ significantly (Table 4.17). A significant ($P < 0.05$) interaction effect was established between the RKN and solarization at 5 cm depth (Appendix XVII). However, the amendment × solarization and nematode × amendment interactions at 5 cm depth were not significant ($P > 0.05$) at the end of solarization (Appendix XVII). Correlation analysis revealed a significant negative relationship ($r = -0.50402$, $P < 0.05$) between temperature and J2 population at 5 cm depth.

Table 4.17: Effect of solarizing soils amended with or without organic amendments on J2 populations in the field test at Chepterwai

Treatments	Initial J2 pop[y]	End of solarization[y]		Mid-season[y]		End of experiment[y]	
		5 cm	15 cm	5 cm	15 cm	5 cm	15 cm
Cm + S	42.3b[y]	5.7c	9.7d	69.7ab	124.7ab	153.7bc	131.3a
Cm - S	26.6c	36.3ab	30.7abcd	104.3a	183.7a	186.7abc	147.7a
Td + S	24.7c	10.7bc	13.7bcd	77.3ab	198.7a	132.3bc	68.7a
Td - S	7.6d	12.0bc	23.0bcd	68.3ab	211.7a	148.3ab	35.3a
Pm + S	25.0a	7.7bc	11.7cd	162.7a	110.0ab	67.7c	76.0a
Pm - S	7.4c	18.0bc	44.3abc	66.7ab	219.0a	167.3abc	69.7a
Co + S	31.7bc	68.7ab	80.3a	203.3a	243.7a	318.3ab	65.0a
Co - S	106.6a	83.3a	102.0a	320.3a	317.7a	578.3a	84.0a
P-value	0.5187	0.0101	0.0456	0.6218	0.5618	0.0471	0.6524
LSD	0.3756	0.6708	0.5997	0.9094	1.0584	0.5069	1.0349

[y] Data are means of three replications. Means on the same column followed by similar letter(s) are not significantly different at $P \geq 0.05$ according to LSD test. Cm - cattle manure; Td - *Tithonia diversifolia* compost; Pm - pymarc; +S - with solarization; -S - without solarization.

At 15 cm, nematode populations differed significantly ($P < 0.05$) between the treatments (Table 4.17). With the exception of solarization × nematode interaction that was significant ($P < 0.05$), those of nematode × amendment and solarization × amendment were not significant at 15 cm (Appendix XVIII). There was a significant negative correlation ($r = -0.4800$, $P < 0.05$) between solarization temperature and J2 population established at 15 cm at the end of solarization.

The weekly maximum soil temperature and the average weekly temperature at 5 cm and 15 cm soil depths are presented in appendices XIX and XX respectively. Comparatively higher soil temperatures were obtained in solarized soils compared to non-solarized soils (Appendices XIX and XX).

Percentage RKN control by solarization after five weeks was highest in amended soils compared with non-amended soils at 5 cm and 15 cm depths. Solarized plots treated with Cm recorded the highest nematode control (84.3 % and 68.4 %), followed by those amended with Pm (57.22 % and 73.6 %) and Td (10.83 % and 40.43 %) at 5 cm and 15 cm depths respectively compared with unamended control (3.6 % and 32.65 % respectively).

The J2 population during the mid season of establishing *S. villosum* did not vary significantly ($P > 0.05$) between the solarized and non-solarized soils and between the treatments at 5 cm and 15 cm soil depths (Table 4.17). However, at the end of the experiment, the J2 population at 5 cm differed significantly ($P < 0.05$) with solarized amended soils having lower populations than non-solarized amended soils (Table 4.17). However, the J2 population at the end of the experiment at 15 cm soil depth did not vary significantly ($P > 0.05$) as shown in Table 4.17.

4.6.3 Root-knot nematode reproduction

The treatments did not differ significantly ($P > 0.05$) on their effect on RKN reproduction (Figure 4.11). Soils amended with Cm, Td and Pm had lower RKN reproduction factor (Rf) that did not differ significantly from the controls (Figure 4.11). In addition, solarized soils with or without amendments had lower Rf compared with non-solarized soils although there was no significant difference established (Figure 4.11).

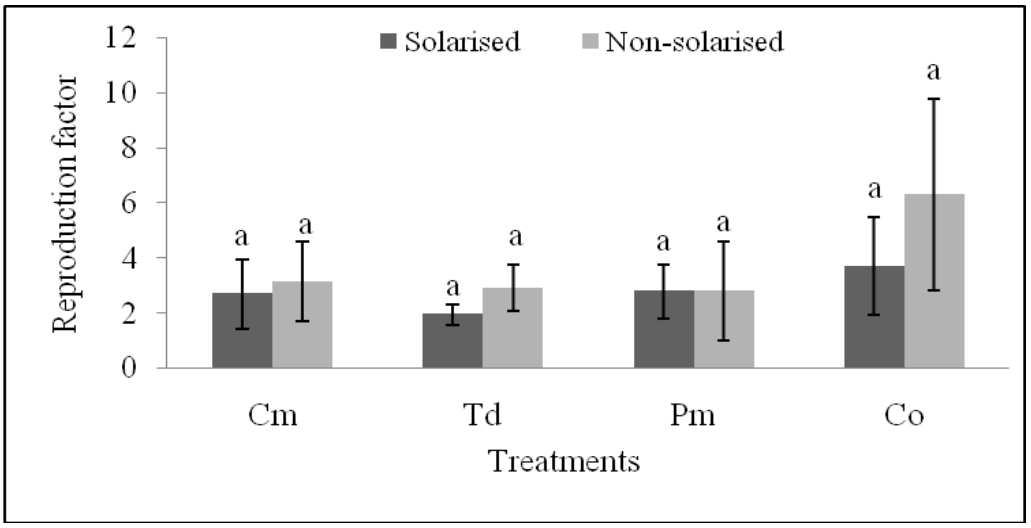

Figure 4.11: Root-knot nematode reproduction on solarized and non-solarized soils with or without cattle manure, *T. diversifolia* compost and pymarc.

Cm - cattle manure; Td - *T. diversifolia* compost, Pm - pymarc and Co - unamended control. Means followed by similar letters are not significantly different at $P \geq 0.05$ according to the LSD test.

4.6.4 Effect of solarizing soils amended with selected organic materials on African nightshade growth characteristics

4.6.4.1 African nightshade shoot height and fresh root weight

Solanum villosum plants grown on solarized and non-solarized amended soils were significantly ($P < 0.05$) taller compared to the control (Table 4.18). Although plants grown on solarized and non-solarized amended soils infested with RKN were shorter than those grown on solarized and non-solarized amended soils without RKN, there was no significant difference ($P > 0.05$) established, except for those grown on solarized and non-solarized soils amended with *T. diversifolia* infested with RKN that were significantly ($P < 0.05$) shorter than those grown on soils without RKN (Table 4.18).

Solarized soils had taller plants that did not differ significantly ($P > 0.05$) from non-solarized soils except for those grown on solarized soils amended with *T. diversifolia* that differed significantly ($P < 0.05$) from non-solarized soils (Table 4.18). Solarization and amendments had significant impact on shoot height although the interaction between solarization × amendments did not have any significant impact on shoot height (Appendix XXI). Correlation analysis revealed a significant negative relationship between the shoot height and the GI ($r = -0.508$, $P < 0.05$) and the EMI ($r = -0.558$, $P < 0.05$) respectively.

Table 4.18: Mean shoot height and fresh root weight of *S. villosum* grown on solarized soils with selected organic materials in the field at Chepterwai

Treatments[z]	Shoot height[y]		Fresh root weight[y]	
	Solarized	Non-solarized	Solarized	Non-solarized
Cm + N	63.86 bc[y]	58.05bcdef	5.63abc	7.88abc
Cm - N	69.53 ab	61.75 bcd	7.64abc	5.01bc
Td + N	64.44 bc	49.11efgh	8.5ab	7.02abc
Td - N	77.27 a	64.84 bc	9.17a	5.59abc
Pm + N	56.41cdef	51.27defg	7.27abc	4.54c
Pm - N	60.73bcde	55.82cdef	7.42abc	4.86bc
Co + N	41.31gh	38.73h	5.76abc	4.87bc
Co - N	46.77fgh	42.81gh	7.69abc	4.89bc
P-value	0.0001		0.7033	
LSD	12.229		3.8401	

[y] Data are means of three replications. Means with the same letter (s) in the same collumn are not significantly different at $P \geq 0.05$ with LSD test.

[z] Cm - cattle manure, Td - *T. diversifolia* compost, Pm - pyrethrum marc, Co - control, +N - with root-knot nematodes and -N - without root-knot nematodes.

The fresh root weight of plants grown on solarized soils with or without nematodes did not differ significantly ($P > 0.05$) from each other (Table 4.18). However, fresh root weight on non-solarized soils varied significantly (Table

4.18). Except for plants grown on RKN infested non-solarized soils amended with Cm that had heavier roots than those grown on solarized soil, all the other treatments did not differ significantly ($P > 0.05$) from each other. Moreover, there was a significant ($r = -0.314$, $P < 0.05$) negative correlation established between fresh root weight and EMI.

4.6.4.2 African nightshade dry shoot biomass

The various treatments differed significantly ($P < 0.05$) on their effect on dry shoot weight (Figure 4.12). Plants grown on solarized amended soils had significantly ($P < 0.05$) heavier dry shoots compared with those grown on non-solarized amended soils except for soils amended with *T. diversifolia* compost and unamended controls that did not differ significantly (Figure 4.12).

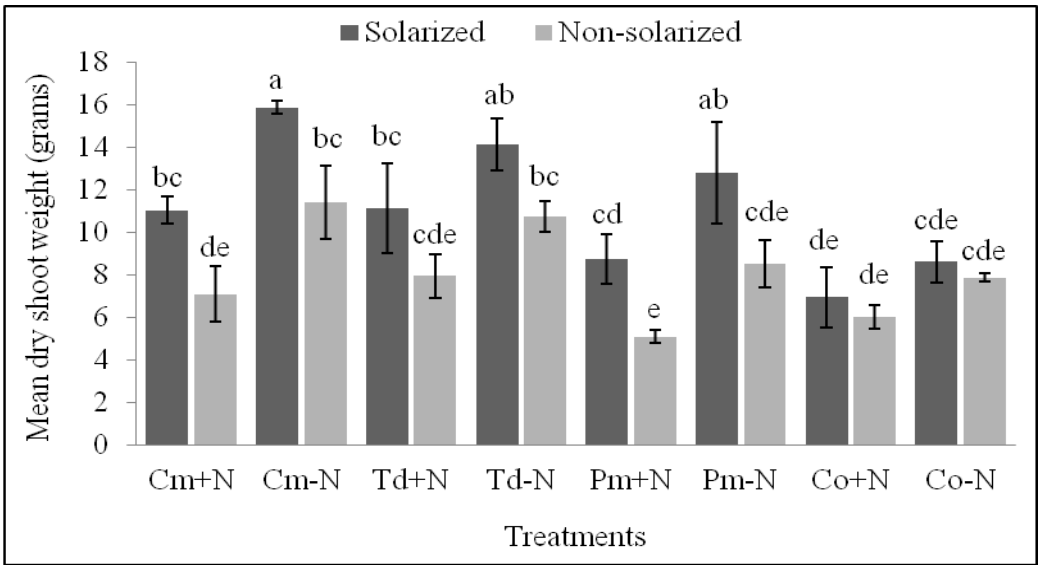

Figure 4.12: Effect of solarization on dry shoot weight of *Solanum villosum* grown on cattle manure, *Tithonia diversifolia* and pymarc amended soils.

Data are means of three replications. Means with the same letter (s) are not significantly different at $P \geq 0.05$ with LSD test. Cm - cattle manure, Td - Tithonia diversifolia, Pm - pyrethrum marc, Co - control, +N - with root-knot nematodes and -N - without root-knot nematodes.

On the other hand, plants grown on nematode infested soils recorded lower dry shoot weight compared to those grown on non-infested soils. Significantly ($P < 0.05$) lower dry shoot weights were recorded on plants grown on RKN infested soils amended with cattle manure on both solarized and non-solarized soils as well as on solarized soil amended with pymarc compared to their controls (Figure 4.12). All the other treatments did not differ significantly ($P > 0.05$) from their controls. Solarized soils amended with pymarc had the highest increase in dry shoot weight (71.57 % and 49.82 %), followed by those amended with cattle manure (55.57 % and 39.33 %), *Tithonia* (40.13% and 31.66 %) and the unamended control (15.81 % and 9.28 %) on both soils with or without nematodes respectively.

The interaction effect of nematode × amendment and nematode × solarization was not significant (Appendix XXII). However, the interaction of solarization × amendment had a significant effect ($P < 0.05$) on the dry shoot weight (Appendix XXII). Correlation analysis revealed a negative relationship between the dry shoot weight and the GI ($r = -0.36827$, $P > 0.05$) (Figure 4.13).

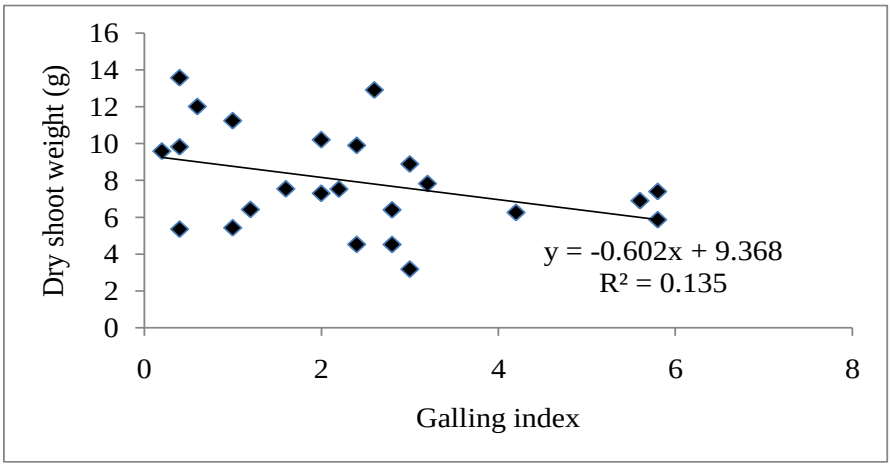

Figure 4.13: Correlation between galling index and dry shoot weight of *Solanum villosum*.

4.6.5 Effect of solarization on soil chemical characteristics

The results for the chemical analysis of solarized and non-solarized amended soil are presented in Table 4.19. Solarization improved soil pH, phosphorus, potassium, calcium, magnesium, iron, zinc and sodium compared with non-solarized soil (Table 4.19). In addition, non-amended solarized soils and cattle manure amended solarized soils had narrower C: N ratio compared to non-solarized soils (Table 4.19). The results also revealed that the amount of copper as well as the concentration of the total nitrogen and total organic carbon in solarized soils was lower than that of non-solarized soils (Table 4.19).

Table 4.19: Chemical properties of solarized soils amended with selected organic materials in field test at Chepterwai

Chemical properties*	Soil treatments							
	Co+S	Co-S	Td+S	Td-S	Cm+S	Cm-S	Pm+S	Pm-S
Soil pH	5.46	5.19	5.46	5.28	5.78	5.41	5.78	5.14
Nitrogen %	0.25	0.27	0.25	0.26	0.26	0.27	0.25	0.24
Org. Carbon %	2.91	3.17	2.86	2.92	3.06	3.30	2.89	2.77
C/N ratio	11.6:1	11.7:1	11.4:1	11.2:1	11.8:1	12.2:1	11.6:1	11.5:1
Phosphorus %	30.0	25.0	25.0	20.0	40.0	25.0	45.0	20.0
Potassium me %	2.16	2.16	2.12	1.54	2.68	2.36	2.59	1.16
Calcium me %	13.9	8.7	9.7	7.7	12.0	10.9	11.9	7.1
Magnesium me %	2.70	2.53	2.71	2.24	3.06	2.52	3.37	2.00
Manganese me %	0.63	0.36	0.55	0.57	0.39	0.71	0.43	0.36
Copper ppm	2.04	2.06	1.83	2.06	1.95	2.03	2.04	2.22
Iron ppm	35.1	33.3	38.0	27.2	40.7	35.3	37.2	21.0
Zinc ppm	4.82	3.88	4.38	3.91	5.41	5.03	5.46	5.00
Sodium me%	1.39	1.29	1.29	0.93	1.53	1.45	1.51	0.73

*Chemical analysis carried out at KARLO-NARL. Co - control; Td - *T. diversifolia*; Cm - cattle manure; Pm - pymarc; +S - solarized; -S - non-solarized soils.

CHAPTER 5: DISCUSSION

5.1 Farmers' awareness, knowledge and management of root-knot nematodes on African nightshades

Higher proportion of farmers growing AFNS for commercial purposes reflects the significance attached to this vegetable as an alternative source of income. In addition, increased awareness of the nutritional and economic benefits of AFNS among the farmers might have resulted to the trend reported herein (Ministry of Agriculture, 2010; Madisa *et al*., 2010). As reported by Abukutsa-Onyango (2007), increased campaigns on consumption of AFNS in both urban and rural set-ups as well as economic returns associated with sale of these vegetables might have also contributed to the increased number of farmers growing the AFNS.

Cultivation of African nightshades in Western and North Rift region of Kenya is a male dominated venture. This might be attributed to the cultural factors that makes men the sole owners of properties and the head of the household. This concurs with studies carried out in Botswana by Obopile *et al*. (2008) and Madisa *et al*. (2010) and Cameroon by Abang *et al*. (2014) revealing the dominance of men in vegetable production in both urban and rural setting.

Majority of the farmers in this study were at their middle age. Young farmers are active in farming and tend to adopt new farming and pest management practices due to access to technology compared with their counterparts (Abang *et al*., 2014). Perhaps this trend might be due to sensitization among the youth about tapping farming as an enterprise. Several organizations in Kenya including Tegemeo group, One-Acre Fund and AGRA are funding small scale farming in the study area, providing farm inputs and

advisory services as witnessed in Kakamega County. This might have attracted younger farmers to venture into farming. Similar findings have been reported in Botswana where younger farmers are funded to carry out agriculture as an enterpreneur (Madisa *et al*., 2010).

Farmers who had gone to school up to primary levels were found to spend more time farming. This might have enabled them to be aware of the pest and disease occurences on their crops. This impacts greatly the level of awareness and management of RKN pests since farmers have access to mobile phones, radio, television, newspapers, Agro-dealers and other farmers that provide information on vegetable pest management (Abang *et al*., 2014). Farmers' education background has been cited to have a significant positive influence in adoption of new technology which aims at alleviating poverty in the rural areas (Das, 1997; Elizabeth and Zira, 2009; Abang *et al*., 2014). Studies have revealed that improved vegetable production strategies requires high level of expertise for effective implementation (Madisa *et al*., 2010). Lapar and Ehui (2003) reported that better educated farmers are generally more open to innovative ideas and new technologies that promote improved vegetable production. Besides, educated farmers have better knowledge of the benefits of adopting improved vegetable production practices and will more likely adopt the practice (Elizabeth and Zira, 2009; Madisa *et al*., 2010).

A large proportion of farmers were not aware of the RKN pest in the surveyed regions except in LM1 AEZ in Kakamega County (Figure 4.2) probably due to lack of organized farmers' groups and visitation by extension workers. This might have compromised the level of farmers' awareness of RKN pest in UM1-UM4 in Bungoma and Uasin Gishu Counties compared to

their counterparts in LM1 in Kakamega County that were aware of RKN pests. Besides, lack of RKN awareness might have contributed to the failure to identify RKN symptoms (Table 4.4) as well as failure to apply control strategies for the RKN (Table 4.5). In addition, farmers in LM1 in Kakamega County were organized in farmers groups and accessed extension services from both the government and non-governmental organizations. This might have improved their knowledge of the RKN pests infesting the AFNS as reflected by a higher proportion of farmers that used AFNS seedlings grown from certified seeds (Table 4.2), or being aware of RKN pests and also being able to identify the symptoms related to RKN (Figure 4.2; Table 4.4). Lack of extension services has been reported to have great influence on farmers' inability to identify vegetable pests and diseases, poor pest management skills and lack of good knowledge of the use of pesticides (Abang *et al*., 2014; Auwal *et al*., 2015).

Most farmers in the study area were unaware of RKN and as such, only a small proportion of them applied Mocap® a chemical nematicide to control RKN. The use of Mocap® in controlling nematodes is worrying due to its toxicity and high persistence in the environment hence its restricted use in Kenya. Misdiagnosis of RKN pest and treatment using wrong approach like arthropod pesticides and cutting infected crops was common in most of the surveyed farms (Table 4.5). Studies carried out in Ghana by Ntow *et al*. (2006) and in Cameroon by Abang *et al*. (2014) reported that farmers apply wrong treatments which puts their health at risk due to chemical toxicity. Farmers have been reported to apply pesticides for quick remedy to their problems (Timper, 2011; Khan *et al*., 2014). These pesticides enter the soil destroying beneficial nematode antagonists. Application of fungicides (Captafol) and bactericides

(Chloropicrin) destroys nematophagous fungi and *Pasteuria penetrans* respectively that antagonize RKN (Timper, 2011).

Farmers also applied animal manure during planting of AFNS as a source of nutrients (Table 4.3). Animal manure especially cattle manure is readily available and accessible to farmers as majority of the farmers own cattle. This is an encouraging move towards the realization of the safe and non-toxic strategies of pest control in the wake of advocating for integrated pest management. Woodash and animal manure have buffering effects on soil pH and also increase soil organic matter. Soil pH impacts RKN distribution and damage in the soil with more damage and reproduction experienced on acidic than alkaline soils (Ferris and Van Gundy, 1979; Sikora and Fernandez, 2005; Asif *et al*., 2015). On the other hand, 6.7 % of the farmers applied woodash, while 3.33 % used crop rotation or uprooted infected crops to control pests and RKN due to lack of enough resources to afford pesticides (Table 4.5). Woodash is readily available as a waste from the fire place and is usually thrown away by most farmers or incorporated into the soil as source of nutrient for planting vegetables. Woodash is not only used to control pests and diseases, improve soil organic matter and texture but also influences the soil pH due to the presence of magnesium and calcium that posses alkaline properties. Application of woodash to control pests allows farmers to harvest the vegetables soon after application of woodash unlike cases where pesticides are applied (Abang *et al*., 2014).

Majority of the AFNS farmers depended on river water for watering their crops with a few others sourcing their water from bore holes, piped water, streams and wells (Appendix X). The variation in the source of water for irrigating their crops could have implications on the damage of African

nightshades by spreading RKN from infected areas (Beije *et al*., 1984). Irrigation water has been reported to contribute to the spread and dissemination of RKN in potato production (Palomares-Ruis *et al*., 2014).

5.2 Root-knot nematode disease incidence, galling index and severity on African nightshades

In this study, root-knot nematodes caused damage to the root system of African nightshades in all the farms from the various agro-ecological zones. An average disease incidence of 94.13 %, galling index (2.9), egg-mass index (2.992) and severity of 3.4 on AFNS grown in the study areas was obtained (Figure 4.3 and Figure 4.4). This might be attributed to poor farming practices like mono-cropping, sharing of seedlings, farm implements and favourable climate and soil in the study area that might have favoured higher nematode reproduction and movement within the soil (Starr *et al*., 1993; Mbogoh *et al*., 2013). Growing susceptible crops like beans, capsicum, potatoes, groundnuts and tomatoes before establishing AFNS as reported by this study, might have favoured RKN reproduction increasing the soil population of RKN leading to higher disease incidence, damage and severity in most farms. It has been demonstrated that poor farming practices like monocropping intensifies RKN damage on crops (Anwar *et al*., 2007; Anwar and McKenry, 2010; Hussain *et al*., 2012). Besides, sharing farm implements and seedlings might have contributed to the spread of the RKN from infected farms to the others (Nchore *et al*., 2012b).

Continuous cultivation of susceptible AFNS (*Solanum villosum*) in UM1-UM4 and intercropping with RKN susceptible crops might have also resulted to higher incidence, disease severity and damage. Besides, a large proportion of farmers did not control RKN in their farms or used wrong strategies like cutting infected crops as revealed in Table 4.5. Monocultivation and intercropping with susceptible hosts to RKN have been associated with an increase in root-knot

nematode infection (Ateeq-ur-Rehman, 2009; Naz *et al*., 2012; Mbogoh *et al*., 2013). Gowen and Channer (1988) reported that phytopathogenic nematodes constrain vegetable production in areas where there is mono-cultivation and succession with nematode susceptible crops like tomatoes, spinach and beans, and where nematode control strategies are currently inappropriate, uneconomical and ineffective. This may pose a major hindrance to commercial and subsistence production of African nightshades.

The study reported severe disease severity in UM1 with mild disease severity in UM2, UM3 and UM4 respectively, while very mild disease severity was reported in LM1 region. The variation in disease severity from severe and mild in UM1-UM4 to very mild disease severity in LM1 might have been due to growth of susceptible AFNS (*S. villosum*) in UM1-UM4 and improved type of AFNS (*S. scabrum*) in LM1 respectively. In addition, the higher disease severity might have been due to low application of organic materials during growth of AFNS as reflected by a higher proportion of farmers using inorganic fertilizer (Table 4.3). Nchore *et al*. (2010) reported varied root-knot nematode damage on indigenous vegetables in different farming systems in Kisii and Trans-Mara areas of Kenya, while Anwar and McKenry (2010) and Naz *et al*. (2012) reported similar findings on grapes and tomatoes respectively in Pakistan. Similarly, Mbogoh *et al*. (2013) and Kimaru *et al*. (2015) reported varied root-knot nematode disease incidence on various African leafy vegetables production systems in Western and Central Kenya respectively. Host genotype has been reported to influence RKN damage and severity with plant genotypes without resistance genes having severe infection (Lopez-Gomez *et* al., 2015).

The variation in disease severity might also be partly contributed by the relatively high temperature in the study areas. This is supported by the work of Jaetzold and Schmidt (1982, 1983); Starr *et al*. (1993) and Mbogoh *et al*. (2013). Higher temperature favour nematode reproduction hence leading to higher disease severity and intensity reported herein (Ateeq-ur-Rehman, 2009; Nchore *et al*., 2010; Maleita, 2011; Hussain *et al*., 2012). Temperatures above the invasion threshold of 16 °C prevail throughout the year in the study areas of Bungoma, Kakamega and Uasin Gishu Counties. This factor might have increased nutrients, nematode movement, rate of growth and reproduction (Ogbuji, 2004; Maleita, 2011) probably causing the higher disease severity, incidences, galling and egg-mass index reported in this study.

The study areas in Lower Midland zone 1 (LM1) and Upper Midland zone 1 (UM1), UM2, UM3 and UM4 agro-ecologic zone, vary in soil type from sandy-loam to clay-loam with poor organic matter probably due to heavy leaching and intensive cropping (Appendices XI-XIII). The sandy-loam and sandy-clay-loam soils prevailing in most farms (Appendices XI-XIII) might have resulted to the higher incidence, galling index and disease severity observed in this study (Figure 4.3 and Figure 4.4).

Farms with sandy-loam and loam in the AEZs in Uasin Gishu and part of Bungoma Counties had relatively higher disease incidence, severity and intensity compared with farms with clay soils (Figure 4.3 and Figure 4.4; Appendices XI-XIII). Clay soil has unfavourable pore size and aeration which might have resulted in poor nematode multiplication and movement. Soil type influences nematode movement as they search for the host, root penetration, reproduction and population build-up in the farms (Prot and Van Gundy, 1981;

Hussain *et al*., 2012). A study by Sikora and Fernandez (2005) reported that soil texture and structure are directly related to water holding capacity and aeration. Naz *et al*. (2012) reported that soils with large amount of sand and gravel favoured root-knot nematode development. These factors are known to influence RKN survival, emergence, disease severity and intensity.

Application of inorganic fertilizers (Diammonium phosphate) during growth of AFNS might have aggravated RKN damage on African nightshades. Diammonium phosphate fertilizers have been reported to affect the soil pH turning the soils acidic. The pH of the soils in the study areas ranged from strongly acidic through slightly acidic in LM1 in Kakamega County and most farms in UM1 to UM4 in Bungoma and Uasin Gishu Counties. However, the pH ranged from near neutral to medium alkaline in those farms where organic materials were incorporated into the soil. This could be due to addition of inorganic fertilizer (DAP) by a large proportion of the farmers in the surveyed region (Table 4.3). The disease severity varied from mild to severe in these Zones while it was very mild in LM1. Correlation analysis revealed a positive relationship between the soil pH and disease incidence and egg-mass index with slightly acidic soils obtained from UM2-UM4. Studies on population buildup at soil pH lower than 5.4 have been reported (Sikora and Fernandez, 2005) with synthetic fertilizers being associated with decreasing RKN antagonistic micro-organism in the soil (Bulluck *et al*., 2002). Additionally, acidic soil is attributed to ionizing free ammonia in water to ammonium ion which is not nematicidal (Oka *et al*., 2000). Soil pH has been reported to have varied effect on RKN distribution in the soil with most RKN species being able to survive and reproduce at pH values ranging from 4.0 to 8.0 (Ferris and Van Gundy, 1979).

Moreover, some species of RKN like *M. javanica* reproduces in soils with a pH range of 6.4-7.0 (Sikora and Fernandez, 2005).

A large proportion of farmers in UM1-UM4 applied cattle manure alone (26.7 %) or combined with sheep manure (3.33 %) during cultivation of African nightshades due to its availability and affordability compared to inorganic fertilizer (Table 4.3). Abukutsa-Onyango (2007) reported that farmers apply animal manure during growth and production of African nightshades. Organic materials supply nutrients to crops making them develop healthy root systems besides releasing toxic phenolic chemicals and gases like ammonia and carbon dioxide that reduces nematode infection. This is supported by a study by Wang *et al.* (2002) and Kimenju *et al*. (2004), who reported the build-up of nematode trapping fungi in soils amended with animal manure compared to inorganic fertilizer.

Organic amendments with a carbon: nitrogen ratio less than 14 especially those of animal origin have been reported to reduce root-knot nematode damage on vegetables (Waceke, 2001; McSorley, 2011). In addition, organic amendments improve soil texture and increases organic matter necessary for nematode management. This agrees with earlier findings by Waceke (2001), Kimenju *et al*. (2004) and McSorley (2011) who reported a reduction in RKN damage following the application of organic materials into the soil during vegetable production that stimulates antagonistic organisms.

The proportion of farmers using inorganic manure to grow AFNS was higher in the surveyed regions. This might have increased the content of potassium and phosphorus in the soil in UM1-UM4 as opposed to LM1 (Figure 4.5). Higher amount of potassium and phosphorus are associated with increase

in RKN reproduction as reflected with the positive correlation relationships established between the galling and egg-mass indices with increasing amount of potassium and phosphorus. This might have resulted to higher galling and egg-mass indices recorded in UM1-UM4 compared to LM1 AEZ that had lower amount of potassium and phosphorus (Figure 4.5).

5.3 Phylogenetic studies of root-knot nematodes on African nightshades based on 18S rDNA

Phylogenetic results show that SSU rDNA sequence can be used to indicate the relationships within *Meloidogyne* species. The study identified the major RKN species occurring in the tropical and sub-tropical regions and also temperate species. *Meloidogyne incognita*, *M. arenaria, M. hapla*, *M. javanica*, *M. lopezi* and *Meloidogyne* spp. isolated from the surveyed areas (Figure 4.7; Table 4.6) did not give identical sequences with the published sequences for the same species. This concurs with studies by Tigano *et al*. (2005) reporting lack of identity between isolates of RKN and the references from the GenBank probably due to molecular heterogeneity in these species.

Meloidogyne incognita, M. arenaria, M. hapla, M. javanica and *M. lopezi* that were identified in this study are the most damaging RKN pests of vegetable crops worldwide. *Meloidogyne incognita, M. javanica* and *M. arenaria* are the most dominant RKN species (Table 4.6) that are widely distributed in the warm and tropical regions while *M. hapla* is restricted to cooler/temperate regions and in upland tropical areas (Maleita, 2011; Onkendi *et al*., 2014). *Meloidogyne incognita, M. arenaria, M. javanica* and *M. hapla* have been reported in Kenya (Muturi *et al*., 2012) as pests of indigenous leafy vegetables, while *M. lopezi* has been reported for the first time in our study.

The higher percentage occurrence reported on *M. incognita, M. arenaria* and *M. javanica* (Table 4.6), reveals that these pests are widespread in the AFNS vegetable production areas. These species are among the most common species in the tropics with *M. incognita* ranked first in relation to its geographical distribution and host range. Besides, *M. incognita, M. arenaria*

and *M. javanica* constitute about 50 % of the total RKN population (Sasser and Carter, 1985) and in the present study the occurrence of this species was found to be between 18.75 and 25.0 % (Table 4.6). On the other hand, three isolates (KE004, KE005 and KE009) identified as *Meloidogyne* spp. could be new species of RKN since it could not compare with any of the RKN and are also among the most widespread in the surveyed region (Table 4.6). The variation in RKN species distribution in the surveyed region may be due to different soil characteristics as reported in Appendices XI – XIII and climate as supported by the work of Jaetzold and Schmidt (1982, 1983); Starr *et al*. (1993) and Mbogoh *et al*. (2013). Therefore, this warrants adoption of strict control measures for their management.

Meloidogyne incognita, *M. javanica* and *M. arenaria* are some of the RKN species believed to have evolved together and sharing apomictic/obligatory mitotic parthenogenetic reproductive strategies (Chen *et al*., 2003; Maleita, 2011). As such, these nematodes could be sharing the same reproductive strategies. On the other hand, *M. hapla* reproduce by facultative meiotic parthenogenesis (automixis) (Tigano *et al*., 2005; Tariq, 2008; Zeng *et al*., 2014). Study by Blok and Powers (2009) revealed that RKN species evolution is related to the mode of reproduction with amphimixis being the ancestral reproductive mode. This therefore, supports clustering of isolates from the surveyed region with different reproductive modes with those from the GenBank.

Although isolates KE005 and KE009 were more divergent from the other RKN species in clade I, they were placed as a sister taxon with *M. incognita*. This suggests that these two species might have diverged recently. Besides, the

divergence might have been due to a higher rate of transitional substitution leading to variation on their genetic materials (DNA) as supported by higher C/T incidence and low polymorphism (Table 4.8 and Table 4.9). Also, different modes of reproduction with the meiotic parthenogenetic groups in clade II and clade III (Figure 4.7) might have led to the divergence reported herein. Mitotic parthenogenetic species of RKN undergoes high metabolic rates or accumulation of DNA replication errors due to shorter generation times that leads to variations within their natural population (Gillooly *et al*., 2005; Subbotin *et al*., 2013).

Isolates KE002 and KE003 were identified as *M. lopezi* while isolate and KE004 was identified as *Meloidogyne* spp. clustering with both *M. ethiopica* that reproduce through obligatory mitotic parthenogenesis (Apomixis) and *M. floridensis* that reproduce through facultative meiotic parthenogenesis (automixis) in clade II (Figure 4.7). *Meloidogyne lopezi* was first reported as a new RKN species on coffee in Costa Rica (Humphreys-Pereira *et al*., 2014). From the surveyed regions in our study, they were identified in LM1 and UM4 zones respectively. These AEZs are characterized with low temperature in some parts of the year that goes as low as 8.4 ^{o}C (Jaetzold and Schmidt, 1983). Perhaps the low temperature might have provided suitable conditions for *M. lopezi* to thrive.

The meiotic and mitotic parthenogenetic isolates in this study were clearly separated except those in clade II where *M. lopezi* clustered with automictic and apomictic RKN species (Figure 4.7). The clustering between these two groups might be supported by the assumption that evolution in *Meloidogyne* species is

related to the mode of reproduction with mitotic pathenogens thought to have multiple origins (De Ley *et al*., 2002; Tigano *et al*., 2005).

5.4 Response of African nightshades to root-knot nematodes

The shoot height and dry shoot weight of various African nightshades varied significantly in both greenhouse and field experiments. The AFNS grown on soils infested with RKN were lighter and shorter compared to those grown on soils without RKN in both greenhouse and field experiments. This might be attributed to among other factors, the damage inflicted on the roots by the RKN causing galling thus hindering uptake of water and essential nutrients for growth of the plants (Anwar and McKenry, 2010; Ononuju *et al*., 2014). Moreover, the food manufactured by plants is redirected to the nematode nursing cells instead of normal plant cell growth thus depriving the plant the essential nutrients for growth (Ononuju *et al*., 2014). A study reported by Jaiteh (2010) shows that heavily galled roots hinder efficient uptake of water and minerals required for proper growth. Deficiency of macro-nutrients reduces plant growth in nematode infested crops and cause chlorosis (Khan and Mukhopadhyay, 2004).

The AFNS grown on nematode infested soils had higher fresh root weight compared to the control. This might be attributed to RKN infestation forming galls and nursing cells that forms nutrient sinks where manufactured food is redirected to feed RKN (Jaiteh, 2010). In addition, RKN also releases growth regulating hormones that causes hypertrophy (Maleita, 2011) resulting to heavier root systems in susceptible AFNS compared to that of tolerant AFNS.

Except for the field test at Chepterwai, the nematode reproduction varied significantly between the AFNS in the greenhouse and field test at Kenyatta University. Although there was no significant difference in RKN damage based on galling index (GI) in the greenhouse, the damage (GI) differed significantly between the AFNS in both field experiments. Both resistant and tolerant AFNS

had lower RKN reproduction compared to susceptible AFNS. Variation in RKN reproduction and host response might be attributed to differences in host genotype that vary in resistance levels probably due to variation in the level of resistance genes. Besides, differences in reproduction rates might be partly due to genetic factor in the host which confers susceptibility or resistance and also genetic differences between the RKN populations (Castagnone-Sereno, 2006). Host resistance to RKN is nematode specific (Khan and Khan, 1991). Variation in nematode species may affect resistance to RKN infection (Lopez-Gomez *et al*., 2015). A plant may be resistant to a specific RKN species due to specific single gene for resistance but when other species of RKN and temperature above invasion threshold prevail, resistance is broken. Cook and Starr (2006) reported that a resistant crop to a specific nematode may be susceptible in fields with mixed nematode populations with different virulence genes enabling them to cause damage to the plants.

The host response of the AFNS varied from resistant to tolerant in the greenhouse and in the field test at Kenyatta University while, at Chepterwai, the response varied from resistant to susceptible. *Solanum eldoretiunum* line MW05 and *S. scabrum* line RV1 that were consistently resistant while *S. sarrarachoides* line MW13 was consistently tolerant in both greenhouse and field conditions. Although *S. americanum* line RC01 and *S. nigrum* line IP03 were resistant in the greenhouse, they were tolerant to RKN in both field experiments, while *S. nigrum* landrace from Kakamega and *S. opacum* were resistant in the greenhouse and field test at Kenyatta University but were tolerant to RKN at Chepterwai. Both *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 were susceptible in the field test at Chepterwai

though they were tolerant in the field test at Kenyatta University. These crops might be possesing traits that are required for nematode parasitism relative to the resistant and tolerant AFNS that could probably have broader absence of the host traits required for RKN parasitism.

Variations in host response of *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 to RKN from resistance or tolerance to susceptibility might be partly due to variation in environmental and edaphic factors as well as the nematode biotypes. In addition, it might be attributed to mixed populations and variation in population density of RKN species between the fields. Variation in host response due to nematode biotypes has been reported in cucurbits (Mukhtar *et al*., 2013b; Lopez-Gomez *et al*., 2015). Studies have reported that moderately resistant/tolerant crops may become susceptible when the population of RKN and the environmental conditions vary (Maleita, 2011). Khan and Khan (1991) reported that tolerant varieties under greenhouse conditions incur significant damage and allow low population build-up under field conditions probably due to variations in nematode species and edaphic conditions. A study by Cook and Starr (2006) reported that tolerant hosts become susceptible when nematode populations and environmental conditions vary. Variations in environmental and edaphic conditions influenced by agro-ecological zones of the field sites and the greenhouse, might have contributed to susceptibility of the AFNS to RKN infection (Mbogoh *et al*., 2013). Favourable environmental and edaphic conditions favour higher nematode reproduction and movement within the soil leading to higher damage to the roots (Starr *et al*., 1993; Maleita, 2011).

Variation in temperature has been reported by Roberts and Omwega (1992) and Maleita (2011) to break RKN resistance when temperature is increased

above 26 ^{o}C. The experimental sites vary in temperature, soils and rainfall amounts as they are located at different AEZs. High temperature above the invasion threshold (16 ^{o}C) prevailed in Chepterwai (Jaetzold and Schmidt, 1983).

5.5 Efficacy of solarizing soils amended with selected organic materials on root-knot nematodes

It is evident from the findings in the present study that solarized soils amended with cattle manure, pymarc and *Tithonia diversifolia* organic materials has a potential as an alternative means of ridding the soils of root-knot nematodes. Transparent polythene material used for solarization allows solar radiation to penetrate but retains the solar heat thus bringing about nematode control (Mokbel and El-Saedy., 2013). Similar results have been reported in Egypt by Bakr *et al*. (2013) and Mokbel and El-Saedy (2013) for controlling RKN on tomato and grapevine respectively, using soil solarization.

Solarization suppressed RKN damage (GI and EMI) as shown in Table 4.15, population (Table 4.16) and reproduction (Figure 4.11) on soils amended with cattle manure, pymarc and *T. diversifolia* organic materials. The GI was significantly suppressed on solarized cattle manure and unamended control compared to non-solarized soils (Table 4.16). Similarly, solarization suppressed EMI on cattle manure and *Tithonia* amended soils. Although solarized soils amended with pymarc suppressed GI and EMI, it did not differ significantly from the non-solarized soil (Table 4.16). On the other hand, the J2 populations were suppressed significantly on solarized soils than non-solarized soils. Moreover, J2 suppression was higher at 5 cm soil depth in solarized soils than non-solarized soils (Table 4.17). This is depicted by significant nematode × solarization and nematode × amendment interactions at the end of solarization and end of experiment (Appendices XVII and XVIII). Suppression of RKN by cattle manure, *Tithonia* and pymarc could be attributed to high pH, organic carbon, nitrogen content, low phosphorus levels and narrow C: N ratio present

in these materials (Appendix II) and in the solarized soils (Table 4.19). *Tithonia*, pymarc and cattle manure have been reported to have lignin and polyphenols that are nematicidal as well as humic and fulvic acids which influences chemical properties of the soil (Jama *et al*., 2000; Reddy, 2013).

Lower levels of total nitrogen and total carbon in solarized soils compared to the non-solarized soils (Table 4.19) and the organic materials before being incorporated into the soil as indicated in Appendix II could be attributed to higher rate of mineralization leading to release of ammonia and Carbon IV Oxide. Higher levels of ammonia and carbon dioxide have been associated with solarization of amended soils and are attributed to control of RKN. Studies by Chen and Katan (1980) reported the release of nitrogen in form of NH_4^+ and NO_3^- in solarized soils.

Solarization narrowed C: N ratio in unamended soils and cattle manure amended soil. Narrow C: N ratio accelerates mineralization of organic matter in soil (Carson and Otoo, 1996; Jenking and Jain, 2010; McSorley, 2011). This is in agreement with studies by Carson and Otoo (1996) in Ghana where higher mineralization and narrower C: N ratio was reported following solarization. Soil with narrow C: N ratio has been reported to reduce nematode damage on crops (McSorley, 2011; Nchore *et al*. 2012b).

Solarizing soils amended with cattle manure, pymarc and *T. diversifolia* organic materials had better effect on nematode control compared to amendments alone. Solarized soils amended with cattle manure and pymarc had the highest nematode control at 5 cm and 15 cm soil depths compared with the control while *T. diversifolia* had the least nematode control. Solarizing soils

amended with organic materials improves their decomposition leading to high temperature that might have killed RKN (Hasin, 2002; Reddy, 2013).

Solarization has been reported to improve the release of collagenase and chitinase enzymes that acts on the cuticle of nematodes and their eggs (Gamliel *et al*., 2000; Reddy, 2013). Suppression of RKN on solarized amended soils compared to the controls might be attributed to increased carbon dioxide, ammonia, phenolic compounds and nematode antagonistic organisms and hence reduced damage to the crops (Gamliel *et al*., 2000; Timper, 2011; Reddy, 2013). Nematode antagonists like *Bacillus* species, *Flavobacterium balustinum*, various *Pseudomonas* species and several fungi (*Streptomyces*, *Penicillin*, *Trichoderma* and *Gliocladium verens*) have been reported to be abundant in decomposed organic materials (Jenking and Jain, 2010).

Although RKN population was suppressed on solarized soils at the end of solarization, the nematodes recovered towards the middle of the cropping season, probably due to growth of susceptible AFNS (*Solanum villosum*). The comparatively shorter (5 weeks) solarization period might have not accumulated enough heat to penetrate deeper into the soil for longer suppression of RKN. The nematodes might have also moved deeper into the lower levels of soil during solarization and only moved back to the upper level after the introduction of susceptible host. Similar studies on recovery of RKN on solarized soils have been reported (McSorley and McGovern, 2000; D`Addabbo *et al*., 2010) with the assumption that some nematodes have a population pool at deeper levels that leads to recolonization (Reddy, 2013).

Suppression of RKN on solarized soils could also be attributed to high temperature during solarization. Heat retained within the polythene mulch

together with that generated by the decomposing cattle manure, pymarc and *Tithonia* might have increased the temperature compared to the non-solarized and non-amended soils (Appendices XIX and XX). Katan *et al*. (1976), Bakr *et al*. (2013) and Reddy (2013) reported that solarizing soils for a period of between four to six weeks raised temperatures to levels that are detrimental to nematode pests. The lethal action of high temperature against the root-knot nematodes could be attributed to distruption and eventual breakdown of the cell membrane (Carson and Otoo, 1996; Bakr *et al*., 2013).

The lethal temperature for eradicating 95 % (LD_{95}) of RKN eggs, egg-masses and juveniles to 46 ^{o}C for 32.4 minutes was established under field conditions in Italy (Ruiz *et al*., 2003). The soil temperature from this study at 5 cm soil depth was way above the LD_{95} for solarized soils (Appendix XIX). This factor might explain the lower nematode population at 5 cm soil depth observed herein. Besides, high temperature is attributed to inactivation of respiratory enzymes of RKN rendering them to be susceptible to toxic gases like ammonia, heat-tolerant antagonistic micro-organisms (Carson and Otoo, 1996; Sharma *et al*., 2002; Ros *et al*., 2008) and nematostatic chemicals affecting reproduction and distribution of the J2 (Bakr *et al*., 2013). In addition, high temperature coupled with low C: N ratio is attributed to accelerating decomposition of organic matter in the solarized soil leading to the production of ammonium and volatile compounds that are trapped between the soil surface and the plastic film thus inhibiting RKN metabolism (Ros *et al*., 2008; McSorley, 2011).

Plants grown on solarized soils were taller and heavier than those grown on non-solarized soils. This might be due to increased breakdown of organic materials in the soil that might have resulted in the release of soluble nutrients

for plant growth (Chen and Katan, 1980; Carson and Otoo, 1996; Reddy, 2013). Solarizing soils amended with organic materials improves release of essential nutrients, microflora beneficial to plant growth and antagonistic organisms to RKN (Viaene *et al*., 2006; Reddy, 2013; Abada *et al*., 2014). There were relatively higher levels of phosphorus, potassium, calcium, zinc, iron, sodium and magnesium in solarized soils compared with non-solarized soils (Table 4.19). Studies by Chen and Katan (1980) and Ahmed *et al*. (1996) reported that soil solarization improved breakdown of organic materials releasing essential nutrients like calcium (Ca^{+2}), magnesium (Mg^{+2}), potassium (K^{+}) making them more available to the plants leading to higher growth. However, solarization did not have much effect on the amount of copper in solarized soils compared to the non-solarized soils. This concurs with studies by Reddy (2013) who reported that solarization did not affect the amount of copper in soil.

Fresh root weight of plants grown on solarized soils amended with cattle manure, pymarc and *T. diversifolia* organic materials was higher compared to those grown on non-solarized soils except for cattle manure amended soils infested with RKN (Table 4.18). This could be attributed to availability of macronutrients and improved uptake of micronutrients availed by solarization (Chen and Katan, 1980; Reddy, 2013). On the other hand, heavier roots on plants grown on non-solarized soil amended with cattle manure and infested with RKN than those of solarized soils infested with RKN (Table 4.18) might be attributed to galling on the root system leading to increased weight. Additionally, heavier roots on solarized soils compared to non-solarized soils might be attributed to reduced RKN population, damage (GI) and reproduction enhancing efficient utilization of the available nutrients for growth.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

- Majority of the farmers lacked knowledge of root-knot nematodes with a large proportion of them being women. Lack of awareness led to misdiagnosis of the nematode pests. In addition, most farmers did not use any strategy to manage root-knot nematodes.
- There was higher disease incidence and severity on African nightshades in Bungoma and Uasin Gishu Counties compared with Kakamega County, thus suggesting their importance as potential threat to AFNS in Kenya.
- Farms where farmers incorporated organic materials like cattle manure and compost had lower RKN disease incidence and severity.
- *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. hapla* and *M. lopezi* were identified from the surveyed regions.
- The host response of AFNS to RKN in this study varied from resistant to susceptible. *Solanum eldoretiunum* line MW05 and *S. scabrum* line RV1, were resistant, while *S. sarrarachoides* line MW13 was tolerant in both greenhouse and field conditions. On the other hand, *S. americanum* line RC01 and *S. nigrum* line IP03 were resistant in the greenhouse, although they were tolerant to RKN in both field experiments, while *S. nigrum* landrace from Kakamega and *S. opacum* were resistant in the greenhouse and field test at Kenyatta University but were tolerant to RKN at Chepterwai. Both *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 were susceptible in the field test at Chepterwai

though they were tolerant in the field test at Kenyatta University. *S. nigrum* landrace from Kakamega

- Solarizing soils amended with cattle manure, pymarc and *T. diversifolia* organic materials resulted to higher shoot height and biomass in *S. villosum*. Pymarc had the highest increase in dry shoot weight (71.57 % and 49.82 %), followed by those amended with cattle manure (55.57 % and 39.33 %), *Tithonia* (40.13% and 31.66 %) and the unamended control (15.81 % and 9.28 %) on both soils with or without nematodes respectively. In addition, solarization improved the efficacy of organic amendments against RKN on *S. villosum* with the highest percentage J2 control being obtained by cattle manure (84.3 %) followed by pymarc (57.22 %), *T. diversifolia* (10.83 %) and unamended controls (3.6 %).

6.2 Recommendations

- There is need to sensitize farmers on root-knot nematode damage on African nightshades. More women should be encouraged to involve in AFNS farming in order to promote food security and alleviate poverty in the rural areas. There is need to educate farmers on proper pest management strategies for diagnosis and control of root-knot nematodes in AFNS.
- To reduce incidence and severity of RKN, there is need to incorporate cattle manure and compost into the soil during production of AFNS.

- There is need to control *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla* and *M. lopezi* that were identified on AFNS from the surveyed region to avert further damage and losses associated with them.
- Further studies are needed to determine the identity of isolate KE005 that could not be achieved in this study.
- Farmers should grow *S. eldoretiunum* line MW05 and *S. scabrum* line RV1 that were resistant to RKN, and *S. americanum* line RC01, *S. nigrum* landrace from Kakamega and *S. opacum* that were resistant in the greenhouse and tolerant to RKN infection in both field experiments to reduce population population build-up and reproduction. Farmers should avoid growing *S. nigrum* from Simlaw Seed Company and *S. villosum* line BG03 that were susceptible in the field test at Chepterwai.
- The tolerant AFNS species should be used in breeding programs for the management of RKN.
- There is need to combine soil solarization with *T. diversifolia* compost, cattle manure and pymarc amendments for RKN management and production of AFNS.
- Further studies should be carried out on different edaphic and climatic conditions where African nightshades are grown to ascertain the efficacy of solarization on organic amended soils against root-knot nematodes.

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APPENDICES

Appendix I: Questionnaire on farmers' knowledge, awareness and pest management practices on root-knot nematodes on African nightshades in Bungoma, Kakamega and Uasin Gishu Counties

Interviewer: Mr. Shem Bonuke Nchore, a student from Kenyatta University, undertaking a Doctor of Philosophy degree programme.

The purpose of this study is to carry out a survey on farmers' knowledge and awareness of the root-knot nematode pests and pest management practices for control of the pest for intervention purposes. The findings from this study will be meant for research only.

1. Personal details

(a) Name of interviewee..... (b) Age.....

(c) Gender..... (d) Education level..... (e) Occupation.....

2. **Location details**: County.....

District..... Location..... Sub location.....

Nearest market/town.....

3. How big is your farm?..... (acres)

4. Do you grow African nightshade (managu) in your farm? Yes ….. No ……

4.1 If Yes, which variety/type do you grow?

1. Traditional …… 2. Improved/Agriculture type …… 3. Others ……..

4.2 Who decides on the variety (s) to be grown?
1 = Head of household 2 = Spouse (wife) 3 = Other (specify)……

4.3 Where do you get the seeds for planting on your farm?

1 = Certified seeds from Agrovets 2 = Own seed
3 = From neighbours 4 = Buys seedlings from neighbours
5 = Sometimes buys seedlings from the market

4.4 Why do you grow this crop?
1. Subsistence …… 2. Commercial use……. 3. Others (Specify)……

4.5 For how long (years) have you been growing these vegetables?.....

4.6 How many crop seasons do you grow Managu in a year? ……...…

4.7 Which planting system do you use? 1 Rows/lines ..... 2 Broadcast .....

4.8 Do you water/irrigate your crops? Yes …….. No ……..…

4.8.1 If Yes, what is the source of the water? ……

5. Which crops were grown before you planted African nightshades on this farm?

6. How long does African nightshade crop take to mature?.....

7. How do you harvest this vegetable crop?.....

8. What challenges/problems do you experience in the production of African nightshade? ……

9. MANAGEMENT OF ROOT KNOT NEMATODES

9.1 Do you apply organic manure on your crops? Yes …… No……

9.1.1 If yes, where is it obtained from? ……

9.2 Do you use other inputs? Yes …… No. …….

9.2.1 If yes, which ones? .....……...

9.3 Are your crops damaged by pests and diseases? Yes …… No ……

9.3.1 If Yes, specify ……..……..

9.4 How do you control pests and diseases on your farm? ……

9.5 Do you know the pests called nematodes? Yes …… No ….……

9.5.1 If yes, what are some of the symptoms/changes they cause on your crops? ……

9.6 How do you control nematodes on your farm ……

9.7 Are there types of African nightshade that you know which are not affected by nematodes?

Yes ……. No …….

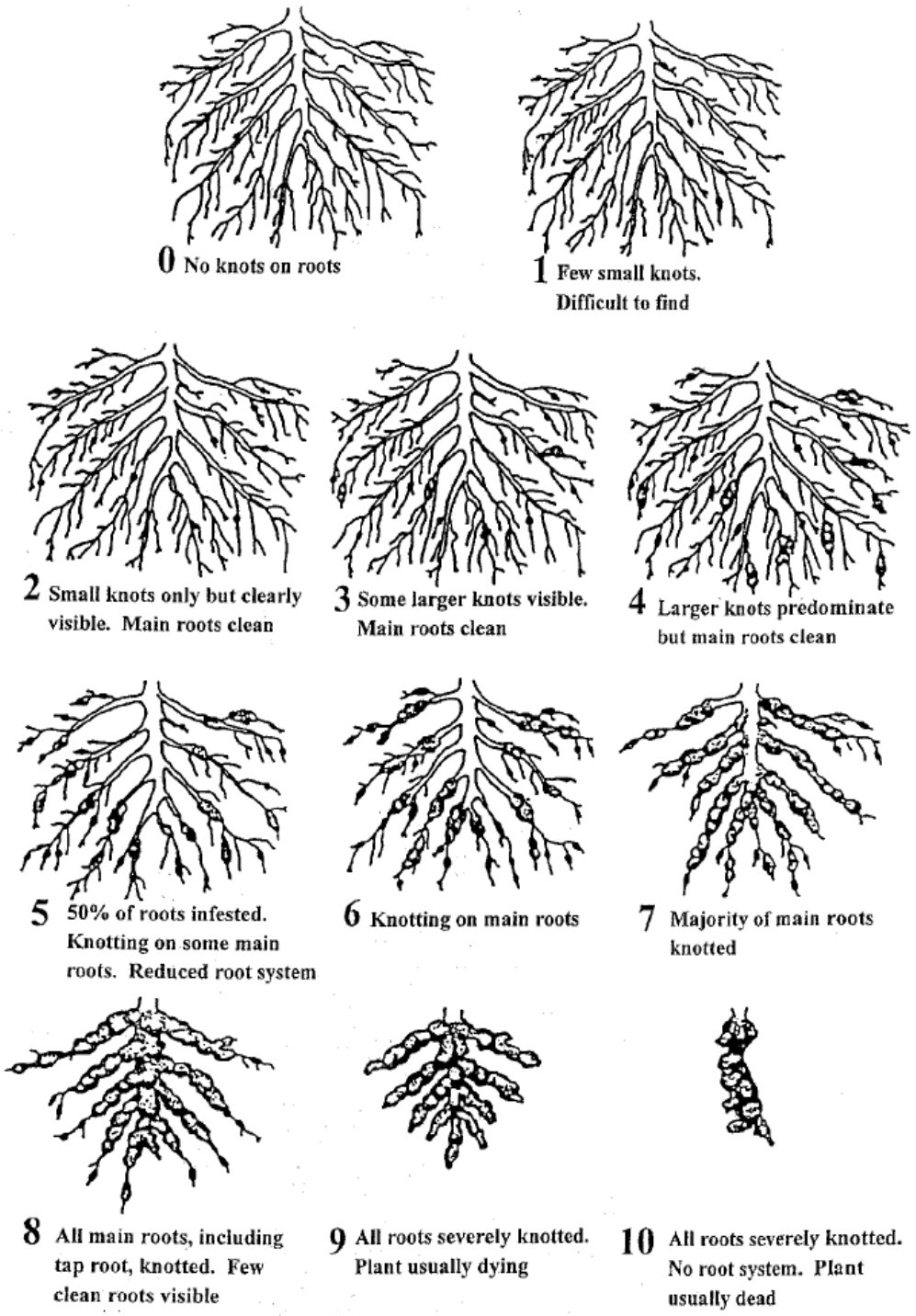
9.7.1 If yes, List them. …….……

Appendix II: Chemical analysis of plant tissue and animal manure used for amending soil in field solarization experiments

Content analysis♦	Cow manure	pymarc	*T. diversifolia* compost
pH-water (1:2.5)	7.34	6.52	6.30
Org. Carbon %	3.70	4.31	4.26
Nitrogen %	1.05	2.45	5.25
C/N ratio	3.5:1	1.8:1	0.8:1
Phosphorus %	0.21	0.44	0.28
Potassium %	1.58	5.49	1.65
Calcium %	0.96	3.95	0.59
Magnesium %	0.08	0.40	0.19
Iron mg/kg	1397	762	2033
Copper mg/kg	3.3	25.0	8.33

♦ Mineral content analysis data test carried out at the Kenya Agricultural and Livestock Research Organization-National Agricultural Research laboratories (KALRO-NARL) in Kenya.

Appendix III: Root-knot galling rating chart for evaluation of *Meloidogyne* infestation according to Bridge and Page (1980)



Appendix IV: General information for the preparation of master mix for a single PCR reaction

Ingredients	Quantity in micro litres (µl)
H_2O	15.4
5× HF buffer	5.0
5 % dimethyl sulfoxide (DMSO)	1.25
Forward primer (20 pmol/µl)	0.7
Reverse primer (20 pmol/µl)	0.7
dNTPs (10 µM each)	0.5
Phusion (enzyme)	0.25
DNA Template*	1.25
Total volume for 1 reaction	**25.05 µl**

* If more or less DNA template was required, the volume of water was adjusted accordingly.

Appendix V: Primers used for identification of root-knot nematode species

Code	Primer sequence 5′-3′	Size (bp)	Specificity and source
1813F 2646R	CTGCGTGAGAGGTGAAAT GCTACCTTGTTACGACTTTT	833	18S rDNA, Holterman *et al*. (2006)
988F 1912R	CTCAAAGATTAAGCCATGC TTTACGGTCAGAACTAGGG	924	18S rDNA, Holterman *et al*. (2006)
1096F 1912R	GGTAATTCTGGAGCTAATAC TTTACGGTCAGAACTAGGG	816	18S rDNA, Holterman *et al*. (2006)

Appendix VI: Optimized PCR conditions for SSU-P66 with primers 1813F/2646R

Step	Temperature (^oC)	Time (hh: mm: ss)	Cycles
Denaturation of the DNA template	98	0:00:30	1 ×
Denaturation of DNA	98	0:00:10	5 ×
Primer annealing	45	0:00:20	
Elongation	66	0:00:30	
Denaturation of DNA	98	0:00:10	35 ×
Primer annealing	54	0:00:20	
Elongation	66	0:00:30	
Final elongation	66	0:07:00	1 ×
Cooling and storage	10	0:00:00	1 ×

Appendix VII: Optimized PCR conditions for SSU-P6640 with primers 988F and 1912R

Step	Temperature (^{o}C)	Time (hh: mm: ss)	Cycles
Denaturation of the DNA template	98	0:00:30	1 ×
Denaturation of DNA	98	0:00:10	5 ×
Primer annealing	45	0:00:20	
Elongation	66	0:00:30	
Denaturation of DNA	98	0:00:10	35 ×
Primer annealing	54	0:00:20	
Elongation	66	0:00:40	
Final elongation	66	0:07:00	1 ×
Cooling and storage	10	0:00:00	1 ×

Appendix VIII: Optimized PCR conditions for SSU-P665640 with primers 1096F and 1912R

Step	Temperature (^{o}C)	Time (hh: mm: ss)	Cycles
Denaturation of the DNA template	98	0:00:30	1 ×
Denaturation of DNA	98	0:00:10	5 ×
Primer annealing	45	0:00:20	
Elongation	66	0:00:30	
Denaturation of DNA	98	0:00:10	35 ×
Primer annealing	56	0:00:20	
Elongation	66	0:00:40	
Final elongation	66	0:07:00	1 ×
Cooling and storage	10	0:00:00	1 ×

Appendix IX: DNA concentration for the PCR products

Sample code	DNA concentration (ng/µl)		
	988F/1912R	1096F/1912R	1813F/2646R
KE001	34.7	27.4	26.5
KE002	10.1	18.3	48.3
KE003	14.8	19.4	13.1
KE004	98.2	68.1	77.1
KE005	36.1	10.9	18.7
KE006	38.4	22.1	35.3
KE007	16.6	21.2	15.5
KE008	15.1	12.0	34.5
KE009	15.4	16.9	36.0
KE010	26.3	12.3	15.4
KE011	85.7	70.1	86.3
KE012	41.2	13.8	16.8
KE013	16.2	15.4	22.2
KE014	30.3	23.0	19.7
KE015	24.9	42.1	34.7
KE016	61.3	43.0	51.6

Appendix X: Proportion of farmers using various sources of water for irrigating African nightshades

Sources of water for irrigating AFNS	**Agro-ecological zones (AEZs)**					
	LM1	UM1	UM2	UM3	UM4	Mean (%)
No irrigation	6.67	3.33	6.67	16.67	13.33	46.7
River	26.67	0.0	10.0	3.33	0.0	40.0
Stream	0.0	0.0	0.0	0.0	3.33	3.33
Borehole	0.0	0.0	0.0	3.33	0.0	3.33
Piped	0.0	0.0	0.0	3.33	0.0	3.33
Well	0.0	3.33	0.0	0.0	0.0	3.33
Chi-square	(χ^2 = 36.509, P = 0.013)					

Appendix XI: Physical-chemical properties of soils from farms in UM1, UM2 and UM3 in Bungoma County

Properties[♦]	**1**[#]	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Soil pH	5.12	4.96	5.35	5.23	4.76	6.22	7.78	4.96	6.22	5.07
Total Nitrogen %	0.31	0.30	0.18	0.18	0.14	0.19	0.16	0.13	0.14	0.19
Org. Carbon %	3.00	3.03	1.73	1.79	1.33	1.91	1.53	1.32	1.39	1.88
Phosphorus ppm	60.0	80	40.0	60.0	15.0	115	80.0	55.0	25.0	50.0
Potassium me %	0.63	1.23	0.59	1.05	0.31	0.43	1.79	0.31	0.71	0.37
Calcium me %	2.0	3.0	2.0	2.6	0.80	2.0	5.0	1.2	2.0	1.0
Magnesium me %	7.59	6.81	4.23	5.02	3.00	4.51	5.51	2.65	2.77	3.08
Manganese me %	0.34	0.47	0.43	0.53	0.45	0.46	0.39	0.43	0.29	0.34
Copper ppm	13.9	13.8	6.83	7.86	3.95	2.39	2.34	2.89	1.87	1.67
Iron ppm	59.5	54.6	57.6	56.4	35.8	86.3	57.4	45.9	47.2	43.6
Texture	CL	SC	C	SCL	SL	SL	CL	CL	SCL	CL

♦ Physical-chemical analysis carried out at Kenya Agricultural and Livestock Research Organization-National Agricultural Research laboratories (KALRO-NARL) in Kenya.

[#] **1-10** = farm 1-10, **C** = Clay, **CL** = Clay Loam, **SC** = Sandy Clay, **SCL =** Sandy Clay Loam, **ppm =** parts per million, **me** = milliequivalents.

Appendix XII: Physical-chemical properties of soils from farms in UM3 and UM4 Uasin Gishu and Nandi Counties

Properties♦	1#	2	3	5	4	6	7	8	9	10
Soil pH	5.32	5.22	5.14	6.90	4.98	4.96	5.81	4.59	5.53	6.53
Total Nitrogen %	0.15	0.25	0.25	0.29	0.21	0.18	0.24	0.18	0.40	0.39
Org. Carbon %	1.51	2.45	2.54	2.88	2.12	1.79	2.32	1.70	3.95	3.91
Phosphorus ppm	10.0	45.0	35.0	85.0	30.0	35.0	50.0	55.0	25.0	110
Potassium me %	1.03	1.49	0.43	0.33	1.29	0.89	0.35	1.09	1.11	1.95
Calcium me %	52.8	4.0	1.4	2.2	3.2	2.6	2.2	2.4	2.8	4.6
Magnesium me %	3.17	3.89	3.82	5.02	3.67	3.0	3.7	2.50	5.69	6.37
Manganese me %	0.26	0.49	0.44	0.29	0.26	0.23	0.18	0.14	0.27	0.40
Copper ppm	1.30	2.04	1.38	1.66	1.00	1.56	1.50	1.72	2.24	2.03
Iron ppm	34.9	35.6	41.7	90.9	46.7	237	82.0	67.6	111	87.0
Texture	L	SL	SL	SL	SCL	SL	L	CL	SCL	CL

♦ Physical-chemical analysis carried out at Kenya Agricultural and Livestock Research Organization-National Agricultural Research laboratories (KALRO-NARL) in Kenya.

1-10 = farm 1-10, **C** = Clay, **CL** = Clay Loam, **L** = Loam, **SL** = Sandy Loam, **SCL =** Sandy Clay Loam, **ppm =** parts per million, **me** = milliequivalents.

Appendix XIII: Physical-chemical properties of soils from farms in LM1 in Kakamega County

Properties[♦]	**1**[#]	**2**	**3**	**4**	**5**
Soil pH	5.08	5.28	4.53	4.58	6.58
Total Nitrogen %	0.17	0.14	0.20	0.13	0.12
Org. Carbon %	1.67	1.30	2.01	1.29	1.14
Phosphorus ppm	35.0	30.0	40.0	35.0	50.0
Potassium me %	0.45	0.39	0.26	0.30	0.37
Calcium me %	1.20	1.20	1.0	0.80	2.2
Magnesium me %	4.08	3.24	4.07	4.06	5.66
Manganese me %	0.29	0.35	0.26	0.28	0.30
Copper ppm	2.81	2.73	2.45	2.80	6.46
Iron ppm	53.5	89.6	110	110	193
Texture	CL	SL	SL	SCL	SL

♦ Physical-chemical analysis carried out at Kenya Agricultural and Livestock Research Organization-National Agricultural Research laboratories (KALRO-NARL) in Kenya.
[#] **1-5** = farm 1-5, **C** = Clay, **CL** = Clay Loam, **SCL =** Sandy Clay Loam, **ppm =** parts per million, **me** = milliequivalents.

Appendix XIV: Small subunit ribosomal DNA sequences for 16 root-knot nematodes infecting African nightshades

> *Meloidogyne javanica* (KE006)
taagttttgattcgttgtattcgatgaaaacctgcgaaaaggactcgattaacaaatggcacttttatttgacctggatct
ttgattggtttaaaatgggataaaatgtggaaaaagcttagaggctagttacatgcaaaaaaagctttgtcaattacg
gaaaaagcgcatttaattagaacaaaaaccacgcggcttcggctgcttcttgttgactcagaataacttagctgacc
gcatggccttgtgccggcggcgtgtctttcaagcgtccactttatcaacttgacgggagcataatcgactcccgtgg
tggtgacggataacggaggatcagggttcgactccggagaaggggcctgagaaatggccactacgtctaaggat
ggcagcaggcgcgcaaattacccactctcggctccaggaggtagtgacgagaaataacgagactgttctctttga
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aatgatttcggttcagttttattggttttacggactgagataatggctaacagagacaaacgggggcatttgtatggtc
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gactcaacacggggaaactcacccggcccggacactgtgaggattgacagattgatagctttttcatgattcagtg
gatggtggtgcatggccgttcttagttcgtggagtgatttgtctggtttattccgataacgagcgagactctaacctac
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aacaggtctgtgatgcccttagatgtccggggctgcacgcgcgctacactggcaaaatcaacgtgcttgtcctacc
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attccaagtaagtgcgagtcatcagctcgcgttgattacgtccctgccctttgtacacaccgcccgtcgctgcccgg
gactgagccatttcgagaaatttggggaccgttgatttaatttttctaaattacttgaagggaaaacccaatttaatcgc
agtggcttgaaccgggcaaagcaaaaaggggggggagaacaaaaaa

> *Meloidogyne hapla* (KE001)
aaccccacagtaaagttaatcgtatttacgagaaaccgcgaacggctcattacaatggccatgatttacttgatcttg
ataatcctaaatggacaactgtggaaaagctagagctaatacactgcactaaagctttgtccttacggaaaagcgca
ttgattagaacaaaaccaagcggcttcggctgcttcttgttgactcagaataactaagctgaccgcatggccaagtg
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acggaggatcagggttcgactccggagaaggggcctgagaaaatggccactacgtctaaggatggcagcagg
cgcgcaaattacccactctcggctcgaggaggtagtgacgagaaataacgaggtcgttctcaaatgaggccggtc
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gtaattccagctctgctaatacatagaattattgctgcggttaaaaagctcgtagttggattcgtgtcgataccctgga
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acaagtttactttgaacaaatcagagtgcttcaaacaggcgtttagcttgaatgatcgtgcatggaataatagaatac
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atttcgagaaacttggagactgttgatctaatttttttaagttactttgagggaaaccaatttaatcgcagtggcttgaac
cg

> *Meloidogyne incognita* (KE014)
tttaatcgtttatcgagaaaccgcgaacggctcattacaatggccattatttacttgatcttgattgtctaaatggataac
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aagggcaccaccaggagtggagcctgcggcttaatttgactcaacacggggaaactcacccggcccggacact
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gtctggtttattccgataacgagcgagactctaacctactaaatagttggtacatactcttagtgtatacagcttcttag
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cgcgcgctacactggcaaaatcaacgtgcttgtcctaccctgaaaggggcgggtaaaccattgaaaatttgccgtg
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cgtccctgccctttgtacacaccgcccgtcgctgcccgggactgagccatttcgagaaatttggggaccgttgattt
aatttttctaaattactttgatggaaaccaatttaatcgcagtggcttgaaccggg

> *Meloidogyne arenaria* (KE012)
ccgcgaacggctcattacaatggccattatttacttgatcttgattgtctaaatggataactgtggaaaagctagagct
aatacatgcactaaagctttgtccttacggaaaagcgcatttattagaacaaaaccacgcggcttcggctgcttcttg
ttgactcagaataacttagctgaccgcatggccttgtgccggcggcgtgtctttcaagcgtccactttatcaacttga
cgggagcataatcgactcccgtggtggtgacggataacggaggatcagggttcgactccggagaaggggcctg
agaaatggccactacgtctaaggatggcagcaggcgcgcaaattacccactctcggctccaggaggtagtgacg
agaaataacgagactgttctctttgaggccggtcatcggaatgggtacaatttaaaccctttaacgagtatcaagca
gagggcaagtctggtgccagcagccgcggtaattccagctctgcaaatacatagaattattgctgcggttagaaag
ctcatagttggattcgtatcgataccttggaacccttcgggtgtctctaggtgttatcgatttatcgtaatgttcggttttg
agtccttaacaggattcttaacaggcattgcaagtttactttgaacaaatcagagtgcttcaaacaggcgttttcgctt
gaatgatcgtgcatggaataatagaaaatgatttcggttcagttttattggttttacggactgagataatggttaacaga
gacaaacgggggcatttgtatggtcccgtgagaggtgaaattcttggaccgtggccagacaaactacagcgaaa
gcatttgccaagaatgtttttattaatcaagaacgaaagtcagaggttcgaaggcgatcagataccgccctttttctga
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tcttccggttccgggggaagtatggttgcaaagctgaaacttaaaggaattgacggaagggcaccaccaggagtg
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gaaagaaattgagcaataacaggtctgtgatgcccttt

> *Meloidogyne arenaria* (KE010)
ccgcgaacggctcattacaatggccattatttacttgatcttgattgtctaaatggataactgtggaaaagctagagct
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gacaaacgggggcatttgtatggtcccgtgagaggtgaaattcttggaccgtggccagacaaactacagcgaaa
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accgtaaacgatgccaactagcgatccgccgatggaaattatattgccttggtggggagcttcccggaaacgaaa
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agctttttcatgattcagtggatggtggtgcatggccgttcttagttcgtggagtgatttgtctggtttattccgataacg
agcgagactctaacctactaaatagttggtacatactcttagtgtatacagcttcttagagggatttgcggcgttcagc

cgaaagaaattgagcaataacaggtctgtgatgcccttagatgtccggggctgcacgcgcgctacactggcaaaa
tcaacgtgcttgtcctaccctgaaaggggcgggtaaaccattgaaaatt

> *Meloidogyne* spp (KE009)

ctctccaaaagagtttataccatgcatgtataagtttaatcgtttatcgagaaaccgcgaacggctcattacaatggcc
attatttacttgatcttgattgtctaaatggataactgtggaaatagctagagctaatacatgcactaaagctttgtcctta
cggaaaagcgcatttattagaacaaaaccacgcggcttcggctgcttcttgttgactcagaataacttagctgaccg
catggccttgtgccggcggcgtgtctttcaagcgtccactttatcaacttgacgggagcataatcgactcccgtggt
ggtgacggataacggaggatcagggttcgactccggagaaggggcctgagaaatggccactacgtctaaggat
ggcagcaggcgcgcaaattacccactctcggctcgaggaggtagtgacgagaaataacgagactgttctctttga
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cgcggtaattccagctctgctaatacatagaattattgctgcggttaaaaagctcgtagttggattcgtatcgatacctt
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aatgatttcggttcagttttattggttttacggactgagataatggttaacagagacaaacgggggcatttgtatggtc
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agttggtacatactcttagtgtatacagcttcttagagggatttgcggcgttcagccgaaagaaat

> *Meloidogyne arenaria* (KE008)

gattaagccatgcatggtataaggtttaatcgttttaacgagaaaccgcgaaccggctcattacaacaaccaccatg
atttacttgatctagattatctaatggataaactgtggaaaaagctagagctaatacatgcacccaaaggctttgtcct
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> *Meloidogyne incognita* (KE013)

gcctgataagttaatcgttaatcgagaatccgcgaacggctcattacaatggccattatttacttgatcttgattgtcta
aatggataactgtggaaaagctagagctaatacatgcactaaagctttgtccttactggaaaagcgcatttattagaa
caaaaccacgcggcttcggctgcttcttgttgactcagaataacttagctgaccgcatggccttgtgccggcggcgt
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> *Meloidogyne lopezi* (KE003)
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ttttccgtaaggacaaagctttagtgcatgtatacccccc

> *Meloidogyne incognita* (KE016)
aatttcccagcatgtataagtttaatcgtttatcgagaaaccgcgaacggctcattacaatggccattatttacttgatct
tgattgtctaaatggataactgtggaaaagctagagctaatacatgcactaaagctttgtccttacggaaaagcgcat
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ggaggatcagggttcgactccggagaaggggcctgagaaatggccactacgtctaaggatggcagcaggcgc
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aatgggtacaatttaaaccctttaacgagtatcaagcagagggcaagtctggtgccagcagccgcggtaattccag
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gtgtctctaggtgttatcgatttatcgtaatgttcggttttgagtccttaacaggattcttaacaggcattgcaagtttactt
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> *Meloidogyne javanica* (KE015)
agtataagttaatcgtttatcgagaaaccgcgaacggctcattacaatggccattatttacttgatcttgattgtctaaat
ggataactgtggaaaagctagagctaatacatgcactaaagctttgtccttacggcaaaaagcgcatttattagaac
aaaaaccacgcggcttcggctgcttcttgttgactctaagaataactttagctgacccgcatggcccttgtgccggg
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gaaaattatatgccgtgcggttaggaaaagcgtctatagtttggattttcgtatttcgataccctctggaaccccttcg
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gagcaattgtcaagtttacttttggaacaaatttcagatgtgccttttcaaaccatggcgtttttttcgcttgaatgatttac
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> *Meloidogyne javanica* (KE007)
tatggagaaaccgggaagggttcattacaatggccctttttttttggttttgattgtttaaaaggataactgggaaaag
gtagagctaatacatgcactaaagctttgtccttacggaaaagcgcatttattagaacaaaaccacgcggcttcggc
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> *Meloidogyne arenaria* (KE011)
cctcgttcacggaaaataattgcaatttccgatcccaatcacggcaaattttcaatggtttacccgccccttcagggt
aggacaagcacgttgattttgccagtgtagcgcgcgtgcagccccggacatctaagggcatcacagacctgttatt
gctcaatttctttcggctgaacgccgcaaatccctctaagaagctgtatacactaagagtatgtaccaactatttagta
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> *Meloidogyne* spp (KE004)
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aatcaacgcgagctgatgactcgcacttactggaaattcctcgttcacggaaaataattgcaatttccgatcccaatc
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agttattctgagtcaacaagaagcagccgaagccgcgtggtttttgttctaataaatgcggtgttccgtaataaattcg
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> *Meloidogyne* spp (KE005)
agcagtataagtttaatcgtttatcgagaaaccgcgaacggctcattacaatggccatgatttacttgatcttgattgtc
taaatggataactgtggaaaagctagagctaatacatgcactaaagctttgtccttacggaaaagcgcatttattaga
acaaaaccacgcggcttcggctgcttcttgttgactcagaataactaagctgaccgcatggccttgtgccggcggc
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accgttgatttaatttttctaaattactttgatggaaaccaatttaatcgcagtggcttgaaccgggcaaagtctaacaa
ggggaaca

> *Meloidogyne lopezi* (KE002)
tgggaattttcgggagtaatacatgcaccaaagctttgtcctctcggaaaagcgcatttattagaacaaaaccacgc
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tgagataatggttaacagagacaaacgggggcatttgtatggccccgtgagaggtgaaattcttggaccgtggcc
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cagataccgccctagtc

Appendix XV: Organic amendment-solarization effect on galling index of root-knot nematode on *S. villosum*

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	10.58250000	3.52750000	4.45	0.0095
Nematode	1	66.74083333	66.74083333	84.16	<.0001
Solarization	1	4.94083333	4.94083333	6.23	0.0174
Nematode*Amendment	3	10.58250000	3.52750000	4.45	0.0095
Solarization*Amendment	3	5.91583333	1.97194444	2.49	0.0766
Solarization*Nematode	1	4.94083333	4.94083333	6.23	0.0174

Appendix XVI: Organic amendment-solarization effect on egg-mass index of root-knot nematode on *S. villosum*

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	14.50000000	4.83333333	24.76	<.0001
Nematode	1	96.33333333	96.33333333	493.41	<.0001
Solarization	1	1.33333333	1.33333333	6.83	0.0131
Nematode*Amendment	3	14.50000000	4.83333333	24.76	<.0001
Solarization*Amendment	3	0.83333333	0.27777778	1.42	0.2526
Solarization*Nematode	1	1.33333333	1.33333333	6.83	0.0131

Appendix XVII: Organic amendment-solarization effect on J2 population in *S. villosum* at 5 cm depth after solarization

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	2292.41667	764.13889	1.61	0.2050
Nematode	1	12096.75000	12096.75000	25.46	<.0001
Solarization	1	6210.75000	6210.75000	13.07	0.0009
Nematode*Amendment	3	2292.41667	764.13889	1.61	0.2050
Solarization*Amendment	3	2421.08333	807.02778	1.70	0.1852
Solarization*Nematode	1	6210.75000	6210.75000	13.07	0.0009

Appendix XVIII: Organic amendment-solarization effect on J2 population in *S. villosum* at 15 cm depth after solarization

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	2204.56250	734.85417	1.19	0.3262
Nematode	1	17290.02083	17290.02083	28.10	<.0001
Solarization	1	6603.52083	6603.52083	10.73	0.0024
Nematode*Amendment	3	2204.56250	734.85417	1.19	0.3262
Solarization*Amendment	3	2357.39583	785.79861	1.28	0.2974
Solarization*Nematode	1	6603.52083	6603.52083	10.73	0.0024

Appendix XIX: Effect of solarizing organic amendments on soil temperature at 5 cm soil depth

	Mean soil temperature (^{o}C) at 5 cm soil depth							
	Solarization				Non-solarization			
Weeks	Cm	Pm	Td	Co	Cm	Pm	Td	Co
1	42.5	43.2	44.8	44.7	38.7	38.3	38.7	37.7
2	41.5	43.7	43.2	42.7	39.2	38.3	38.5	38.5
3	42.3	43.5	44.3	43.3	37.8	38.8	38.5	39.7
4	43.7	45.0	47.2	46.8	39.3	39.7	39.8	39.7
5	47.5	47.8	50.2	49.2	42.3	43.2	44.2	44.2
6	46.0	48.2	49.5	48.2	41.5	42.0	44.3	43.0
AWT*	43.9	45.2	46.5	45.8	39.8	40.1	40.7	40.5
WMT**	47.2	48.2	49.0	48.2	41.7	42.0	43.0	36.7

* AWT- Average weekly temperature
** WMT- Weekly maximum temperature

Appendix XX: Effect of solarizing organic amendments on soil temperature at 15 cm soil depth

	Mean soil temperature (^{o}C) at 15 cm soil depth							
	Solarization				Non-solarization			
Weeks	Cm	Pm	Td	Co	Cm	Pm	Td	Co
1	35.5	36.7	37.8	37.3	31.2	29.7	30.7	31.0
2	36.3	38.3	36.5	37.3	31.0	31.5	31.0	31.7
3	35.3	37.5	36.0	37.0	30.5	31.0	30.8	32.2
4	36.0	37.3	36.8	36.8	30.7	30.7	29.8	30.5
5	38.2	37.8	40.8	39.2	30.8	31.7	32.0	32.0
6	36.2	38.7	39.8	38.8	30.5	29.8	30.7	30.3
AWT*	36.3	37.7	38.0	37.7	30.8	30.7	30.8	31.3
WMT**	39.0	41.2	40.7	40.3	32.8	32.7	33.0	33.3

* AWT- Average weekly temperature
** WMT- Weekly maximum temperature

Appendix XXI: Organic amendment-solarization effect on shoot height of *S. villosum* grown on solarized amended soils

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	3600.454600	1200.151533	24.13	<.0001
Nematode	1	595.302533	595.302533	11.97	0.0014
Solarization	1	629.300833	629.300833	12.65	0.0011
Nematode*Amendment	3	209.657933	69.885978	1.41	0.2576
Solarization*Amendment	3	194.890833	64.963611	1.31	0.2879
Solarization*Nematode	1	0.007500	0.007500	0.00	0.9903

Appendix XXII: Organic amendment-solarization effect on dry shoot weight of *S. villosum* grown on solarized amended soils

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Amendment	3	127.8532417	42.6177472	10.17	<.0001
Nematode	1	125.6905565	125.6905565	30.00	<.0001
Solarization	1	84.5352083	84.5352083	20.18	<.0001
Nematode*Amendment	3	13.0266	44.342227	1.04	0.3884
Solarization*Amendment	3	50.1581898	16.7193966	3.99	0.0152
Solarization*Nematode	1	0.4654454	0.4654454	0.11	0.7409