ASSESSMENT OF ROOT KNOT NEMATODES (*Meloidogyne* spp.) PREFERENCES TO COMMONLY GROWN TOMATO VARIETIES IN MVOMERO DISTRICT

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EXTENDED ABSTRACT

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are among the serious biotic constraints to tomato growers in Mvomero District but relatively overlooked. The study aimed at improving tomato production by identifying tomato genotypes that are resistant to RKN. The specific objectives were to: - (i) assess current status of RKN in Mlali, Doma and Dakawa. (ii) identify species of RKN affecting tomato in Mlali, Doma and Dakawa (ii) screen available tomato varieties for their inherent resistance to RKN.

Multisage sampling procedure using semi-structured questionnaires was adopted to collect data from 100 randomly selected respondents in Doma (33), Mlali (33) and Dakawa (34) wards. Data were collected on socio-economic status, RKN awareness, tomato varieties grown, seed sources and yield. Data from questionnaires were summarised in excel sheet then imported to the statistical package for social sciences (IBM SPSS Statistics version 25) for the analysis of descriptive statistics and correlation. One fifty root and rhizosphere soil samples (75 root and 75 rhizosphere soil) were also collected from tomato fields with plants at flowering/fruiting stage which showed stunting, chlorosis and wilting signs. Fifteen fields located at least 1 km apart in Kipera (2), Mkuyuni (3), Doma B (3), Kihondo (2) and Wami Dakawa (5) villages were sampled. Soil and root samples (five samples of each per field) were collected about 25 cm deep using a shovel, packed in bags, labelled and transported to Tanzania Agricultural Research Institute (TARI) - Kibaha Nematology laboratory. Fourteen (14) tomato cultivars and three (3) tomato breeding lines were screened for resistance to RKN. They were inoculated with 500 second stage juveniles (J2) per pot in a screenhouse pot experiment organised in a randomised complete block design (RCBD) at TARI – Kibaha. The experiment had seventeen treatments (i.e., fourteen tomato cultivars and three breeding lines) with four replications. Data collected were plant height, fresh shoot and

dry weights, fresh root weight, galling, RKN population in root and soil. The reproductive factor was calculated as the ratio of the total RKN population (root + soil) to the inoculated population. The data were subjected to the analysis of variance (ANOVA) using GenStat for windows 20th edition (VSN International, Hemel Hempstead, UK) and treatment means were compared using least significant difference (LSD) at 5% significance level.

Chi-square test ($\chi 2$) detected a significant difference ($p = 0.01$) in awareness on RKN across categories of respondents. However, 59% of respondents were not aware of RKN. Pearson's correlation coefficient determined significant correlation between farming experience and yield of tomato ($p < 0.001$), Farming experience and awareness of RKN ($p < 0.001$) and between yield and awareness on RKN resistant tomato varieties ($p < 0.008$). Popular tomato varieties grown by farmers were Rio Grande (14%), Cal J (18%), Roma (10%) and Tanya (16) while hybrids were Imara F1 (19%), Assila F1 (15%), Jarrah F1 (2%), Zara F1 (3%), Kipato F1 (2%) and Anna F1 (2%). Soil and root samples revealed a significant prevalence ($p = 0.002$) and incidence ($p < 0.001$) of RKN. A total of 27 populations were successfully cultured and identified. Laboratory examination of perineal patterns morphology alongside molecular techniques targeting the mitochondrial DNA (mtDNA) and the internal transcribed spacer (ITS) region was done. Results revealed *Meloidogyne incognita* (18 populations), *M. javanica* (8 populations) and *M. arenaria* (1 population) through a combined perineal pattern morphology of adult female of *Meloidogyne* spp. and PCR - species specific primers. It was found that Anna F1, Assila F1, Imara F1, AVTO1703 and ATO1424, were significantly ($p < 0.001$) resistant to RKN. Tengeru 97 was tolerant while Tanya, Kiboko, Tengeru 2010, Duluti, Meru, Rio Grande, Cal J, Zara F1, Kipato F1, Jarrah and AVTO1704 were susceptible.

The study has revealed that 59% of respondents in Mvomero District were not aware of RKN problem. Three *Meloidogyne* spp. i.e. *M.incognita*, *M.arenaria* and *M. javanica*

were identified in Mvomero District with *M. incognita* being the dominant specie. This finding aids in better understanding of the diversity and prevalence of these important *Meloidogyne* spp. Tomato cultivars Anna F1, Assila F1, Imara F1 and tomato breeding lines AVTO1424 and AVTO1703 showed significant resistance to *Meloidogyne* spp. From the above findings, awareness campaign is recommended to enhance tomato growers' understanding how to manage RKN in their farms. Moreover, farmers in Mvomero district should be encouraged to grow tomato cultivars Anna F1, Assila F1, Imara F1 which revealed significant resistance to *Meloidogyne* spp. There is a need for extensive assessment of RKN to enable understanding of *Meloidogyne* spp. occurrence and distribution in other parts where tomato is grown under intensive production.

DECLARATION

I, SAMWELI OMBAELI do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

…… **……**

Samweli Ombaeli **Date**

MSc. Crop Science Candidate

The above declaration is confirmed by;

…… **……**

Dr. H. D. Mtui **Date**

(Supervisor)

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First of all, I thank God for guidance, help and blessings throughout my life,

I would like to express my sincere appreciation and thanks to Dr. Hosea D. Mtui of SUA and Dr. Nessie Luambano of TARI Kibaha, who supervised this work and made me grow as a scientist.

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The World Vegetable Centre (AVRDC) for supplying some of the tested plant materials,

All tomato growers in Mvomero district who were involved in the interview.

DEDICATION

I dedicate this work to Almighty God who guided me in my study; nothing could make my academic dream come true without God wishes.

My parents, Eliminata M. Kavishe and Ombaeli S. Msuya for all their prayers for me which sustained me this far,

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Centre
COI	Cytochrome oxidase region I
COII	Cytochrome oxidase region II
cv	Cultivar
DNA	Deoxyribonucleic acid
ESA	East and Southern Africa
F1	First filial generation
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GS	Gall scores
GoK	Government of Kenya
GPS	Global Positioning System
Ha	Hectare
ITS	Internal Transcribed Spacer Region
J2	Second juvenile stage of nematode
kb	kilobase
kg	kilogramme
km	kilometre
l-rRNA	Large ribosomal Ribonucleic acid
LSD	Least significant difference
LSU	Larger subunit
m.a.s.l	Metres above sea level

MSc	Master of Science
OPV	Open pollinated variety
PCR	Polymerase chain reaction
Pf	Final population of nematode
PhD	Doctor of Philosophy
Pi	Initial population of nematode
PPN	Plant parasitic nematodes
p-value	Probability value
qPCR	Quantitative polymerase chain reaction
r	Correlation coefficient
Rf	Reproductive factor
RFLP	Restriction Fragment Length Polymorphism
RKN	Root - knot nematode
rRNA	Ribosomal ribonucleic acid
SADC	Southern African Development Community
SCAR	sequence characterised amplified region
SEM	Scanning electron microscopy
SPSS	Statistical package for social science
SSA	Sub-Saharan Africa
SSU	Smaller sub unit
St	Saint
SUA	Sokoine University of Agriculture
t	Tonne
Taq	*Thermus aquaticus*
TARI	Tanzania Agricultural Research Institute
TASC	Tanzania Agriculture Sample Census

TOSCI	Tanzania Official Seed Certification Institute
UK	United Kingdom
URT	United Republic of Tanzania
USA	United States of America
UVL	Ultra violet light
VF	Verticillium and Fusarium wilts resistant variety
µl	Microlitre
%	Percent
&	And
/	Per
<	Less than
=	Equal to
>	Greater than

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop that gains great popularity over time due to its diversified use (Tay*e et al*., 2013). It is a reliable source of vitamin A, B, C, E and minerals such as K, P, Ca, Mg, and Fe hence play a vital role towards ensuring food security and nutrition (Olaniy*i et a*l., 2010; Bhowmi*k et a*l., 2012; Brasesco, *et a*l., 2019). An antioxidant lycopene present in tomato reduces cancers and development of atherosclerotic cardiovascular disease and osteoporosis (Raiol*a et a*l., 2014).

The centre of tomato domestication and diversification is Mexico (Peralt*a et al*., 2005). This Solanaceous crop was introduced to Africa in the 16th century (Lind*a et a*l., 2016; Muimba-Kankolongo, 2018). It is grown worldwide on more than 5 million hectares with a production of approximately 180 million metric tons (FAOSTAT, 2019). About 62% of world production comes from Asia while the remaining 12% is from Africa, 12.6% is from Europe and 13.2% from North, Central and South Americas (FAOSTAT, 2019). In sub-Saharan Africa (SSA), tomato is grown for income generation, local use as well as for export (Dub*e et a*l., 2020). Tomato production in Tanzania has been fluctuating over years (Fig.1.1)

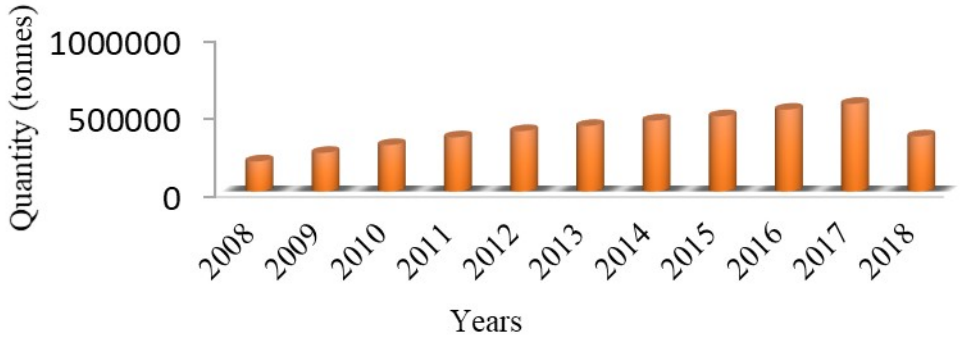

Figure 1.1: Tomato production trend in Tanzania from 2008 to 2018

Source: FAOSTAT

Tomato production is challenged by biotic (pests including root-knot nematodes) and abiotic (extreme temperatures, water and nutrient stresses) factors. Root-knot nematodes (RKN) (*Meloidogyne* spp.) were named after their characteristic root galling symptoms they induce in host plant roots (Plate 1.1). The term '*Meloidogyne*' is the derivative of Greek words implying pear-shaped female (Karssen and Moens, 2006). *Meloidogyne* spp. are classified under the phylum Nematoda, class Chromodorea, order Rhaditida and family Hoplolaimidae (Decraemer and Hunt, 2013).

Plate 1.1: Typical symptoms (galls) of RKN infection on the tomato root system

Source: This Study.

The life cycle of RKN comprises six stages starting with an egg, four juvenile stages and the adult male and female (Eisenback, 2014). Females lay approximately 500 eggs in a gelatinous matrix produced from their rectal glands (Mohamed *et a*l., 2017), which protect eggs from environmental stresses and attack by microbes (Moens *et a*l., 2009). When conditions are favourable, eggs hatch to produce the first juvenile stage (J1) and first moult occurs within the egg resulting to the second stage juvenile (Mohamed *et a*l., 2017). The second stage juvenile (J2) is infective and commences instantly to find the host to feed upon (Doncaster and Seymour, 1973). Juveniles penetrate in the zone of root elongation by using their stylet and migrate intercellularly, initially to the root apex and then to the vascular cylinder, and establish their permanent feeding sites called "Giant cells" (Castagnone-Sereno and Danchin, 2014; Escobar *et a*l., 2015). The sedentary J2 undergo three consecutive moults to become adults. Pear-shaped females remain sedentary, producing large egg masses that are extruded in a gelatinous matrix out of the root while vermiform males migrate out of the root (Abad *et a*l., 2003). A distinct sexual dimorphism occurs at the adult stage, with vermiform, mobile males and pear-shaped, sedentary females (Castagnone-Sereno and Danchin, 2014). This sedentary endoparasitic, obligate and polyphagous genus of plant parasites penetrate plant roots and seizes the nutrients of the host for their own advantage (Karssen *et a*l., 2013; Sauce*t et a*l., 2016).

The developmental lifecycle of *Meloidogyne* spp. can take three weeks to 2 months depending on factors such as temperature, moisture and availability of an appropriate host (Taylor and Sasser, 1978). According to Ploeg and Maris (1999), the life cycle of *M. incognita* completes in 20 and 63 days at 30°C and 16°C respectively. The genus *Meloidogyne* comprises many different species including; *M. Arenaria, M. incognita, M. javanica* and *M. hapla* (Jones *et a*l., 2013; Cetintas and Cakmak, 2016).

Meloidogyne spp. Can be identified using morphological features, isozymes, differential host range and molecular diagnostics (Hunt and Handoo, 2009). Among morphological features, perineal patterns i.e. fingerprint-like cuticular pattern of adult female around the vulva-anus region is the frequently used identifier (Hunt and Handoo, 2009) (Fig. 1.2). However, the weakness of this method is that it cannot be used for closely related *Meloidogyne* spp. due to significant variations within the same population (Zijlstr*a et a*l., 2000). Therefore, perineal patterns examination alongside molecular analyses can result into more reliable results (Cunh*a et a*l., 2018).

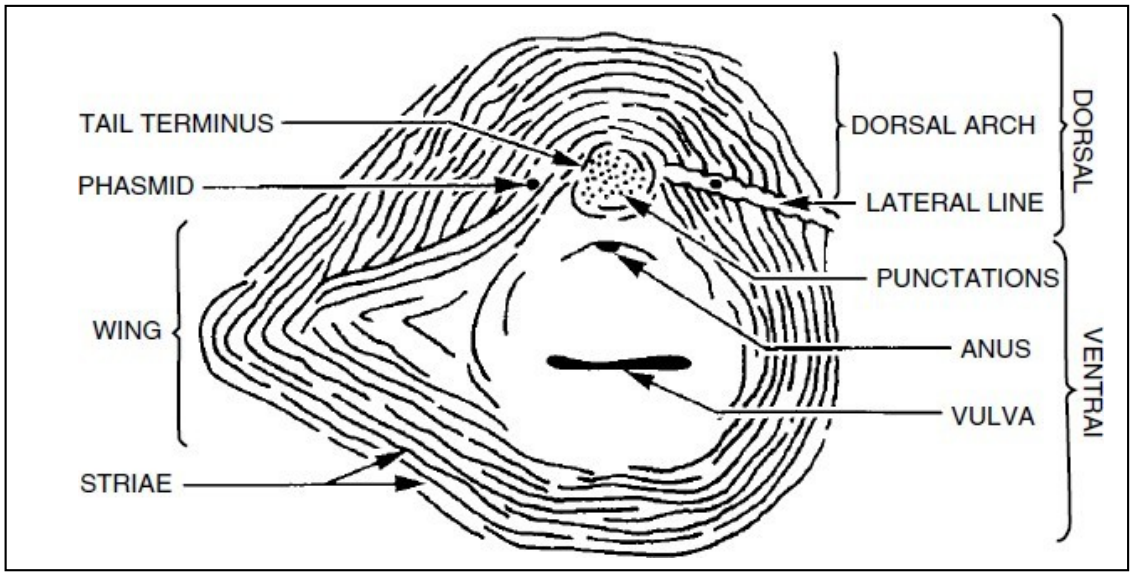

Figure 1.2: Female perineal patterns of *Meloidogyne* spp.

Source: Karssen and Moens (2006)

Molecular diagnostics employs PCR-based methodologies to facilitate species diagnostics and phylogeny within the genus *Meloidogyne* (Cunh*a et a*l., 2018).

A range of management options have been adopted to manage *Meloidogyne* spp. The options embrace fallowing or flooding infested land, use of non-hosts or resistant crop planted in planned cropping systems, disinfection or protection of planting material from infected area, application of nematicides to soil and/ or foliage, the use of organic soil

amendment and destruction of roots from residual crop (Ibrahi*m et a*l., 2018; Çatalkaya and Devran, 2019). Recently, biological control techniques have been used with various rates of successes in controlling RKN ; Ratika and Dey, 2014; Luamban*o et a*l., 2019). However, the techniques are rarely practiced by poor resource smallholder scale farmers. Synthetic nematicides are quick acting in controlling RKN. However, they are non-biodegradable, expensive and cause environmental pollution. In view of the aforementioned, there is a need to search for alternative nematode control methods which may lower tomato production cost while conserving the environment.

Old tomato varieties including Rio Grande and Roma VF are still widely grown in Tanzania because of their important quality traits such as general appearance and extended shelf life (Kagiraneza, 2007). However, such varieties are susceptible to many diseases (Dhaliwal, 2001). Commercial varieties carrying the *Mi* gene for RKN resistant have been used successfully to manage *M. incognita, M. javanica* and *M. Arenaria* (Sei*d et a*l., 2015). But it is known that the gene may breakdown especially in tropics due to high temperature (Sei*d et a*l., 2015). Thus, identifying those varieties currently available in the market through screening and use them to control *Meloidogyne* spp. can counteract the use of expensive nematicides. Moreover, screening available tomato cultivars on their levels of susceptibility and tolerance against RKN may provide room to wide range of options to produce tomato at minimum cost due to reduction of nematode management costs.

1.2 Problem Statement and Justification

1.2.1 Problem statement

The average yield of tomato in Tanzania has been estimated to be 15.4 t/ha (FAOSTAT, 2019) which is lower than the world average of 35.9 t/ha (FAOSTAT, 2019). Low tomato yields in Tanzania have been attributed to various constraints including pests (Mater*u et*

*a*l., 2016). According to Mamiro *et al*. (2015), abiotic factors that limit tomato production in Tanzania include fusarium wilt (*Fusarium oxysporum*), early blight (*Alternaria solani*), septoria leaf spot (*Septoria lycopersici* and RKN. Root-knot nematodes have been reported to cause yield loss in numerous agricultural crops, including tomato (Sei*d et a*l., 2015; Okorl*ey et a*l., 2018). The annual yield loss caused by *Meloidogyne incognita* in tomato in East and Southern Africa (ESA) is estimated at 20.6 % (Talwan*a et a*l., 2015). They feed on the root system of plants by means of a stylet. They are disseminated in planting materials such as seedlings, rootstocks, tubers, rhizomes and corms. However, their damage and control have been receiving inadequate attention, leading to increased tomato yield loss (Talwan*a et a*l., 2015).

Severely RKN infested tomato plants may stunt, wilt, or die before reaching maturity (Singh and Khurma, 2007). However, these symptoms of RKN in tomato plants can be mistaken for other problems such as yellowing, stunted growth and wilting that may result from environmental stress, other pests or nutrient deficiency.

Nematode management in the tropics has been lagging behind compared to the level of expertise attained in the developing world. This is associated with poor awareness and the failure to clearly define nematode impact and establish sustainable nematode management for smallholder farms (Sikor*a et a*l., 2018).

Poor awareness and management of RKN have resulted to declining tomato productivity in major tomato producing areas including Mlali, Doma and Dakawa Wards in Morogoro, Tanzania. Moreover, most tomato varieties grown by small holder farmers in Tanzania are susceptible to RKN. Nevertheless, the degree of susceptibility to RKN is not yet known

making it difficult to decide which variety to recommend to farmers for growing in RKN hotspot areas.

1.2.2 Justification

Crop productivity need to be improved to cope with the increasing food demand (FAO, 2017). Tomato plays a critical role in meeting human nutritional requirements, creation of employment, generation of income and foreign currency earnings (Karuk*u et a*l., 2016; Wanjoh*i et a*l., 2018). To achieve its aforementioned roles, the crop needs to be protected from pests including RKN to improve its productivity.

Correct identification of RKN species affecting tomato in Mlali, Doma and Dakawa was the crucial initial step in suggesting appropriate management tactics against this menace. The use of tomato varieties resistant to RKN is a practical option, particularly for small-scale farmers with limited resources (Nono-Womdi*m et a*l., 2002). This is because, they can counteract the use of expensive nematicides and other costly management practices (Cortad*a et* al., 2008).

Therefore, screening of tomato varieties grown by small holder farmers was done to assess levels of resistance to RKN and provide viable recommendations to growers. In Tanzania, there is a significant move towards vegetable production in screenhouses. With such a high investment, tomato with resistance to nematodes will be a strategic management practice for greenhouse production. Thus, this study focused on assessing farmers' awareness on RKN, identifying species of RKN affecting tomato in Mlali, Doma and Dakawa and screening tomato varieties for their inherent RKN resistance.

The study findings will contribute in updating records of the occurrence distribution of *Meloidogyne* species and designing sound management programmes to boost tomato productivity in the study areas and other areas with similar situation.

1.3 Research Objectives

1.3.1 Overall objective

Identifying tomato genotypes that are resistant to RKN

1.3.2 Specific objectives

i. To assess current status of RKN in Mlali, Doma and Dakawa.
ii. To identify species of RKN affecting tomato in Mlali, Doma and Dakawa.
iii. To screen available tomato varieties for their inherent resistance to RKN.

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CHAPTER TWO

Paper One

2.0 THE STATUS OF TOMATO ROOT-KNOT NEMATODES (*Meloidogyne* spp.) IN MLALI, DOMA AND DAKAWA WARDS IN MVOMERO DISTRICT, MOROGORO REGION

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2.1 Abstract

Root-knot nematodes (RKN) are among serious biotic constraints to tomato growers in Tanzania but relatively overlooked. This necessitated to conduct a survey to capture pre-existing knowledge of growers on RKN in Mvomero Disrict, Morogoro, Tanzania. Multistage sampling procedure was used to obtain respondents for interiew. Semi-structured questionnaires were used to collect data from 100 randomly selected respondents in Mlali (33), Doma (33) and Dakawa (34). Data were collected on socio-economic status, awareness of respondents on RKN, tomato varieties grown, seed sources and yield. Results indicate that there was a significant variation ($p = 0.01$) in awareness on RKN across categories of respondents. However, 59% of respondents were not aware of RKN. There was a significant correlation between farming experience and yield of tomato ($p < 0.001$), Farming experience and awareness of RKN ($p < 0.001$) and between yield and knowledge on RKN resistant tomato varieties ($p < 0.008$). Popular tomato varieties

grown by farmers were Rio Grande (14%), Cal J (18%), Roma (10%) and Tanya (16%) while hybrids were Imara F1 (19%), Assila F1 (15%), Jarrah F1 (2%), Zara F1 (3%), Kipato F1 (2%) and Anna F1 (2%). Seventy five root and 75 soil samples of tomato plants at flowering/fruiting stage showing stunting, chlorosis and wilting signs were collected from fields located at least 1km apart in Mlali, Doma and Dakawa. Samples were collected about 25 cm deep using a shovel, packed in sterile plastic bags, labelled and transported to TARI Kibaha Nematology laboratory for RKN analysis. Results revealed the significant prevalence ($p = 0.002$) and incidence ($p < 0.001$) of RKN. Despite the occurrence and damage caused by RKN in tomato in the study areas, only one percent of respondents recognised RKN as a serious problem. Awareness campaign on RKN will facilitate farmers' consciousness of their existence and management.

Keywords: Awareness, *Meloidogyne* spp., Mvomero, prevalence, severity, survey.

2.2 Introduction

Tomato (*Solanum lycopersicum* L.) is among important vegetable crops in Tanzania. It is grown on approximately 40 820 ha with a total production of 627 788 tonnes (FAOSTAT, 2019). Morogoro region has the highest annual production of 155 745 tonnes (URT, 2017). The average tomato yield attained by smallholders in Tanzania varies from 2.2 to 16 t/ha (Msogoya and Mamiro, 2016). The estimated average yield of tomato in Tanzania is 15.4 t/ha (FAOSTAT, 2019), which is significantly lower than the average yield of 20.4 t/ha attained in Kenya and much less than the world average yield of 35.9 t/ha (FAOSTAT, 2019).

Tomato production is constrained by biotic factors including, unavailability of quality seeds, pests and abiotic factors such as moisture stress, heat, low soil fertility and lack of

appropriate cultural practices (Teste*n et* al., 2018; Palilo, 2019). Root-knot nematodes are among the serious biotic factors which cause low tomato productivity in Tanzania (Mamir*o et al*., 2015). They initiate galls in tomato roots which tend to appear about 25 days post infection (L*u et a*l., 2020). According to García and Sánchez-Puerta (2012), successful host infection depends on the particular interaction between a specific nematode species and race and a specific plant species and cultivar. Moreover, the level of damage generally depends on factors such as the nematode species, host plant, crop rotation regime, season and soil type (Moen*s et a*l., 2009; Olsen, 2011).

Despite the economic loss they cause, they are relatively overlooked because their occurrence is poorly understood by small scale farmers (Janss*en et a*l., 2017). The symptoms of RKN in inflicted tomato plants such as yellowing, stunted growth and wilting may also be attributed to environmental stress or nutrient deficiency (Coyn*e et a*l., 2018). Poor awareness and management of RKN have resulted to declining tomato productivity in major tomato producing areas in Tanzania (Missanga and Rubanza, 2018). Furthermore, there is limited information associated with RKN prevalence and how they are perceived by tomato growers in Mvomero District. Therefore, this study focused on assessing the prevalence, incidence and farmers awareness of RKN affecting tomato in Mlali, Doma and Dakawa wards in Mvomero District.

2.3 Materials and Methods

2.3.1 Description of study area

The survey was conducted in October, 2019 in farmers' tomato fields in Mlali (06° 57′ 0″ South, 37° 32' 0" East), Doma (7° 14' 0" South, 37°13' 0" East) and Dakawa (6° 26' 0" South, 37° 42' 0" East) Wards of Mvomero District (6˚14' 8.2212" South, 38˚ 41' 37.4928" East) in Morogoro Region, Tanzania. These wards are located at an altitude of

358 - 570 m above sea level. Annual rainfall is between 600 mm and 1000 mm. The average temperature ranges from 18 - 30 °C. The dominant soil type in Mlali and Doma is sandy loam (Mbogoni and Ley, 2008), while at Dakawa is sandy clay loam (Mbag*a et a*l., 2017).

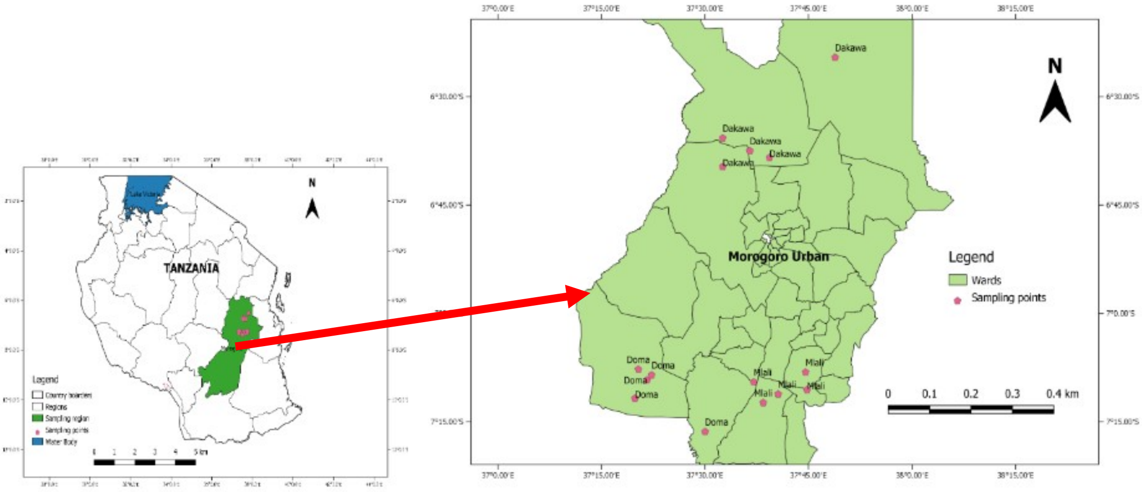

Figure 2.1: Map of Morogoro region indicating areas where sampling for plant parasitic nematodes was done

Source: This study

2.3.2 Sample size and sampling

The sample size for respondents was calculated according to Anderso*n et* al. (2014);

$$n = \frac{p(1-p)Z^2}{E^2} = \frac{0.93(1-0.93)1.92^2}{0.05^2} = 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, n = required sample size, Z = confidence level at 95% (standard value of 1.96), p = estimated proportion of an attribute (average % of tomato farmers in a population of horticultural farmers in the district) and *E* = margin of error at 5%.

Secondary data were obtained from existing sources including journal articles and government reports. The distribution of respondents was as follows; Mlali (33), Doma, (33) and Dakawa (34) making a total number of 100 respondents.

A field survey involving multistage sampling technique was used in selecting respondents (Schreinemachers *et a*l., 2015). Mvomero district was purposively selected among one of the major tomatoes producing district of Morogoro. The second stage entailed purposive selection of three wards namely Mlali, Doma and Dakawa based on high tomato production. Five villages (Kipera, Mkuyuni) in Mlali, (Doma B, Kihondo) in Doma and Wami Dakawa in Dakwa wards were purposively selected. The third stage entailed a simple random selection of respondents (Mwatawal*a et a*l., 2019). This was done with the assistance from Village Extension Officers. This method is cost-saving and guarantees representativeness of the target population (Anderso*n et* al., 2014).

Respondents were interviewed using semi-structured questionnaire. Personal interview was done because it enables real-time response and clarification of questions. Tomato growers who had no tomatoes in their fields were also involved in the interview to capture their awareness on RKN.

2.3.3 Data Collection

2.3.3.1 Farmers awareness of RKN

Baseline data were collected in October 2019 through face-to-face interviews and filled in pre-tested semi-structured questionnaires. Face to face interview was done as it allows real time clarification of questions. Coloured pictures of RKN infested tomato plants showing root galling symptoms were used to confirm farmers' awareness (Lutu*f et a*l., 2018).

2.3.3.2 Occurrence of root-knot nematodes

One hundred and fifty (75 root and 75 rhizosphere soil) samples were collected from fifteen fields of about 0.1 ha each in Kipera (2), Mkuyuni (3), Doma B (3), Kihondo (2)

and Wami Dakawa (5) villages. Tomato fields with plants at flowering/fruiting stage located at least 1 km apart were selected for assessment. Tomato plants which showed symptoms such as chlorotic leaves, wilting and stunted growth were marked. Thereafter, five symptomatic tomato plants per field were carefully uprooted to a depth of approximately 25 cm using a hand shovel (Coyn*e et al*., 2014). Collected root and rhizosphere soil samples from a single sampling point were loaded in sterile plastic bags bags and labelled to make a sample. Labelled samples were packed in a cool box and transported to TARI -Kibaha Nematology Laboratory. In the laboratory, the roots samples were gently cleaned of embedded soil in running tape water. Cleaned roots were visually observed for presence of galls and scored for galls using RKN galling scale of 1 (no galling) to 5 (severe galling) (Coyn*e et a*l., 2018).

The frequency of occurrence (prevalence) and incidence of nematode were determined according to Khan and Ahamad (2020) as follows;

$$\text{Prevalence} = \frac{\text{Number of fields with RKN infestation in one location}}{\text{Total number of field surveyed in the same location}} \; x\; 100 \ldots\ldots\ldots (2)$$

$$\text{Nematode incidence} = \frac{\text{Number of plants galled in one field}}{\text{Total number of plants sampled in the same field}} \; x\; 100 .. (3)$$

Surveyed fields were geo-referenced using the Global Positioning System (GPS) (Garmin-etrex 10, Taiwan).

2.3.3.3 Extraction and quantification of root - knot nematodes from field samples

Individual sub samples of 5 g from cleaned roots were weighed on a digital weighing scale (Wagtech, ADG 600L, Wagtech International Ltd, UK). Weighed roots were then cut into approximately 1 cm pieces using a pair of scissors, macerated in a laboratory blender (Waring Commercial, HGB2WTS3, Torrington, CT, USA) at 18 000 revolutions per minute for about 5 seconds and incubated at room temperature (25 - 28 °C) for 24 hours. Similarly, soil sub samples of 100 cm^3 were measured in beaker. All roots and soil

subsamples were individually subjected to the modified Baermann technique as described by Coyn*e et al*. (2014) to extract nematodes. Nematodes in 2 ml aliquot from each of soil and root samples were counted three times each with the aid of a tally counter and stereo microscope (Leica DM 2500, Leica Microsystems, US) at 10× magnification. The means of the counted nematodes were used to estimate root and soil populations of RKN. Morphological identification to genus level was done parallel with counting using identification key and descriptors illustrated by Mai and Lyon (1975).

2.4 Data Analysis

Quantitative and qualitative data from completed questionnaires were coded before subjected to statistical analysis using Statistical Package for Social Sciences software (IBM SPSS Statistics version 25). The descriptive statistics analysed included frequencies and percentages. To make statistical inferences, contingency chi-square tests were computed at $p \leq 0.05$ levels of significance across categories. Pearson's correlation coefficients were calculated to determine linear relationships amongst variables.

Nematode population counts for roots and soil were normalised by transforming them to $\log_{10}(x+1)$ before they were subjected to analysis of variance (ANOVA). For each, nematode counts were assessed separately from roots and soil sample. Means were compared by Least Significant Difference (LSD) at $p \leq 0.05$ using GenStat for Windows 20th Edition (VSN International, Hemel Hempstead, UK).

2.5 Results

2.5.1 Social background of respondents

There was no significant difference ($\chi^2 = 0.86$; $p = 0.911$) across surveyed wards on the gender of respondents (Table 2.1). However, it was observed that male dominated female in tomato production across the study areas. Majority of respondents (89%) were males

while (11%) were females. The age of tomato growers differed significantly ($\chi 2 = 9.503$; $p = 0.05$) across categories of the surveyed wards (Table 2.1). Forty one percent of respondents interviewed were aged between 18 and 35 years while 39% ranged between the age of 36 - 45 years and the rest (20%) were more than 45 years old. Also, there was no significant variation ($\chi^2 = 3.205$; $p = 0.527$) in education level of respondents was observed across categories of the surveyed wards (Table 2.1). The majority of tomato growers (77%) had primary school education whilst 33.0% had secondary school education. Moreover, there was significant difference ($\chi^2 = 13.638$; $p = 0.009$) across categories of surveyed wards in farm size of tomato growers (Table 2.1). Majority of tomato growers (63%) had farm size in the range of 0.4 – 0.8 ha whilst 24% had farm size less than 0.4 ha and the rest (13%) had farm size bigger than 0.8 ha. Furthermore, the farming experience of respondents across categories of the surveyed wards varied significantly ($\chi^2 = 15.388$; $p = 0.04$). Majority of tomato growers (50%) had farming experience ranging from 2 – 5 years while (46%) had farming experience of more than 5 years and the rest (4%) had farming experience of less than 2 years (Table 2.1).

Table 2.1: Social - economic characteristics of respondents (n = 100)

Social - economic characteristics	Ward			Mean (%)	df	χ^2	p-value
	Mlali (n = 33)	Doma (n = 33)	Dakawa (n = 34)				
Sex							
Male	87.9	90.9	88.2	89	2	0.186	0.911
Female	12.1	9.1	11.8	11			
Age							
18 – 35	48.5	36.4	38.2	41.0			
36 – 45	39.4	27.2	50.0	39.0	4	9.503	0.05
> 45	12.1	36.4	11.8	20.0			
Education level							
Primary school	78.8	81.8	70.6	77.0	2	15.388	0.527
Secondary school	21.2	18.2	29.4	33.0			
Farm size							
< 0.4 ha	42.4	12.1	17.6	24.0			
0.4 – 0.8 ha	54.5	63.6	70.6	63.0	4	13.638	0.009
> 0.8 ha	3.0	24.2	11.8	13.0			
Farming experience							
< 2 years	6.1	0	5.9	4.0			
2 - 5 years	24.2	69.7	55.9	50.0	4	15.388	0.004
> 5 years	69.7	30.3	38.2	46.0			

*df = degree of freedom, $\chi 2$ = Chi-Square test, $p \leq 0.05$ shows significant difference

2.5.2 Tomato seed sources

Seed sources varied significantly ($\chi^2 = 10.615$; $p = 0.031$) across the surveyed wards. However, the majority (81%) of growers were sourcing tomato seeds from agro-inputs dealers (Table 2.2). Other tomato growers were using their own saved seeds (6%) whereas others were using both own saved seeds and those from agro-inputs dealers (13%).

Table 2.2: Sources of tomato seed sources across the wards *(n = 100)*

Sources of seeds	Wards			Mean (%)	df	$\chi 2$	p-value
	Mlali (n =33)	Doma (n =33)	Dakawa (n =34)				
Own saved	0.0	0.1	8.8	6.0			
Agro dealers	97.0	66.7	79.4	81.0	4	10.615	0.031
Agro dealer+own saved	3.0	24.2	11.8	13.0			

*df = degree of freedom, $\chi 2$ = Chi-Square test, $p \leq 0.05$ shows significant difference,

2.5.3 Cropping patterns

There was no significant difference (χ^2 = 1.982); p = 0.421) in cropping patterns across the surveyed wards. However, the survey revealed that the majority of tomato growers (68.9%) were practicing crop rotation (Fig. 2.2). They rotated tomato with beans, cowpeas, watermelon, paddy, onions, pumpkins, maize, okra, sweet pepper, amaranth, Chinese cabbage and hot pepper.

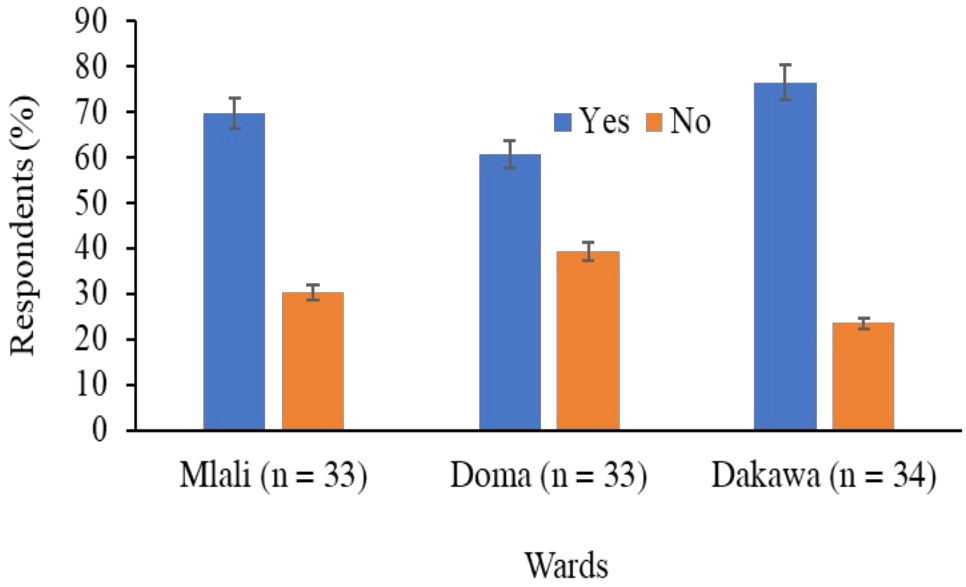

Figure 1.2: Crop rotation across the surveyed wards

2.5.4 Management measures for RKN in tomato fields

There was significant difference (χ^2 = 17.006; p = 0.03) on RKN control measures used by growers. Control measures adopted to mitigate RKN were; applying chemicals (3%), adding manure (4%), crop rotation (20%), uprooting (8%). The survey however, revealed that 59% of respondents did not know any method that could be used to manage root-knot nematodes (Table 2.3).

2.5.5 Ranking of pest challenges faced by tomato growers

The distribution of ranking of the most important pest of tomato was similar (χ^2 = 7.119; p = 0.524) across categories of respondents (Table 2.3). According to the growers

interviewed, the most important challenge was *Tuta absoluta* (72%) followed by wilting (16%), fungus (8%), RKN (1%) and virus (1%) (Table 2.3).

Table 2.3: The awareness of tomato growers on RKN *(n = 100)*

RKN awareness	Wards						
	Mlali (n = 33)	**Doma (n =33)**	**Dakawa (n = 34)**	**Mean (%)**	**df**	**χ2**	**p-value**
Yes	21.2	57.6	44.1	41.0	2	9.226	0.01
No	78.8	42.4	55.9	59.0			
Symptoms							
Root galling	30.3	15.2	32.4	26.0			
Stunting	3.0	0.0	0.0	1.0	6	14.770	0.022
Wilting	27.3	6.1	5.9	13.0			
Don't know	39.4	75.8	61.8	59.0			
Control							
Adding manure	6.1	0.0	5.9	4.0			
Chemical	3.0	6.1	0.0	3.0			
Uprooting	27.3	3.0	11.8	14.0	8	17.006	0.03
Rotation	24.2	12.1	23.5	20.0			
Don't know	39.4	78.8	58.8	59.0			
Pest problems							
Tuta absoluta	69.7	75.8	70.6	72.0			
Bacteria	18.2	12.1	23.5	18.0	8	7.119	0.524
Fungi	9.1	12.1	2.9	8.0			
RKN	3.0	0.0	0.0	1.0			
Viruses	0	0	2.9	1.0			

*df = degree of freedom, χ2 = Chi-Square test, $p \leq 0.05$ shows significant difference

Yield of tomato did not differ significantly ($\chi2 = 3.867$; $p = 0.424$) across categories of respondents. Majority of respondents (52%) were obtaining a yield of 10 – 19 t/ha while a yield of 1- 9 t/ha and >19 t/ha were attained by 24% and 24% of tomato growers, respectively (Table 2.4).

Table 2.4: Tomato yield across categories of surveyed wards (n = 100)

Yield	**Wards**						
	Mlali (n =33)	**Doma (n =33)**	**Dakawa (n =34)**	**Mean (%)**	**df**	**χ2**	**p-value**
1-9 (t/ha)	21.20	27.30	23.50	24.0			
10-19 (t/ha)	48.50	60.60	47.10	52.0	4	3.867	0.424
>19 (t/ha)	30.30	12.10	29.40	24.0			

*df = degree of freedom, χ2 = Chi-Square test, $p > 0.05$ shows non-significant difference

2.5.6 Correlation between variables

The results presented in Table 2.5 indicate that farming experience was positively correlated with awareness on RKN and yield of tomato $r(98) = 0.38$, $p < 0.001$. Likewise, the awareness of RKN was positively correlated with the yield of tomato $r(98) = 0.21$, $p = 0.04$.

Table 2.5: Pearson's correlation coefficients used to assess correlation among farming experience, tomato yield, awareness on RKN and their related p-values (n = 100)

	Farming experience	Awareness on RKN	Yield
Farming experience	1		
Awareness on RKN	0.258**	1	
	0.001		
Yield	0.381**	0.205*	1
	0.001	0.04	

*Correlation coefficient values are significant at $p \leq 0.05$ level

2.5.7 Popular tomato cultivars grown in Mvomero District

Popular tomato varieties grown by farmers included open pollinated varieties (OPV) and hybrids. Open pollinated varieties were Cal J (18%), Tanya (16%), Rio Grande (14%), and Roma (10%) while hybrids were Imara F1 (19%), Assila F1 (15%), Jarrah F1 (2%), Zara F1 (3%), Anna F1 (2%) and Kipato F1 (2%) (Fig. 2.3).

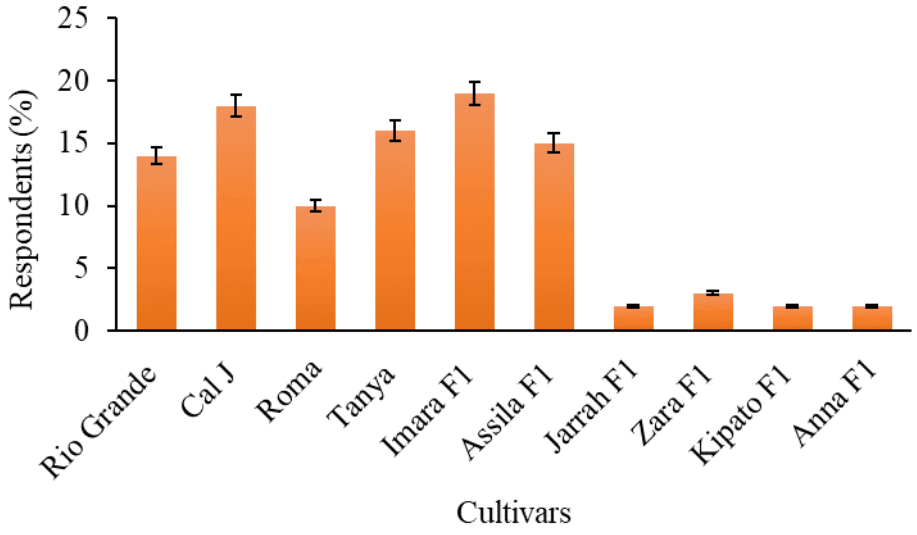

Figure 2.3: Popular tomato cultivars grown in Mvomero District

2.5.8 Incidence, prevalence, severity and population densities of *Meloidogyne* spp.

The mean RKN incidence varied significantly ($p = 0.002$) among the study areas (Table 2.6). There was also a significant difference ($p < 0.001$) in RKN prevalence along the studied locations. Doma had the highest RKN incidence and prevalence (Table 2.6). The lowest RKN incidence and prevalence were observed in Dakawa (Table 2.6). Galling scores for RKN on tomato roots did not vary significantly ($p = 0.06$) between the studied areas. Moreover, RKN populations per 100 cm^3 of soil did not differ significantly ($p = 0.074$) between studied locations (Table 2.6). There was no significant difference ($p = 0.809$) in RKN population per 5 g of roots (Table 2.6).

Table 2.6: The mean incidence, prevalence and gall scores (GS) of Meloidogyne *spp. in the study area*

Location	Incidence	Prevalence	(GS)	RKN/100 cm^3 soil (transformed)	RKN/5g (root transformed)
Dakawa	16.00a	36.00a	2.30	0.524	0.49
Doma	32.00b	80.00c	2.30	0.631	0.36
Mlali	20.00a	64.00b	1.80	0.220	0.44
LSD (5%)	9.15	13.32	ns	Ns	Ns
p-value	0.002	< 0.001	0.06	0.074	0.809

*Means within a column followed by the same letter are not significantly different ($p \leq 0.05$); LSD = Least significant difference; GS = gall score; ns = non-significant

2.6 Discussion

Tomato production in the study area was dominated by males, denoting that they were the majority of tomato growers. The finding is in line with that of who reported 86.4% and 13.6% of male and female tomato farmers, respectively in Mvomero District. This could be linked to fact that men are the principal landowners in the farming community. Furthermore, Masunga (2015) reported the dominance of men (66.7 %) over women (33.7 %) engaged in tomato production in Musoma Municipality, Tanzania. Other reason could be deduced from the fact that tomato production is a capital and labour intensive activity and men have greater access to capital than women as reported by Anan*g et al*. (2013).

Majority of tomato growers (41%) in the study area were aged between 18 - 35 years suggesting that a large segment of youths in the study area were actively participating in tomato production. It is also an indication that there may be a high potential for boosting tomato production in the area. This could be due to the reason that tomato is a high value crop which attracts youths. A similar result on dominance of youths was reported by who did a study concerning tomato production in Mvomero District. However, the results by Mwang*i et a*l. (2015) indicated that 56% of tomato growers interviewed in Mwea sub-county in Kenya who aged between 20 - 40 years were actively participating in tomato production.

The highest percentage of respondents had primary school education and a few had secondary education. The results imply that tomato growers in the study area could understand and implement basic management practices against pests that affect tomatoes. The results are in line with that of Mwatawal*a et a*l. (2019) who reported most respondents (94%) were with primary education in Mvomero district.

The average farm size for tomato production was 0.4 to 0.6 ha. The finding suggests that the majority of farmers involved in tomato production are smallholder farmers. Mwatawal*a et a*l. (2019) also reported an average farm size under tomato production of 0.56 ha in Mvomero District, Tanzania. The finding further concurs with that of Moranga (2016) who reported the average farm size of 0.4 ha under tomato in Kenya.

This study revealed that most farmers were not using hybrid seeds. Limited adoption of hybrid varieties could be the result of limited income/capital for smallholder farmers' which lead to preference for open pollinated varieties as an alternative to hybrid varieties which are relatively expensive. Majority of growers were sourcing seeds from agro-dealers indicating that they had access to quality seeds, which in turn may increase their production levels. Other results (Hanani, 2016; Ochil*o et a*l., 2019) have indicated that profitable farmers are capable of accessing quality inputs for crop production.

This study found that respondents with more than five years of farming experience in tomato production were aware of RKN. Benjami*n et al*. (2017) reported that knowledge of the prominently visible pests is normally known by the farmers given the extended period of cultivation. This is similar to the findings reported by Janat*i et a*l. (2018) indicating that RKN associated symptoms can easily be identified by farmers due to the presence of characteristic galls on the root systems. However, only one percent of respondents declared RKN as a serious problem. This could mean that the problem is overlooked and respondents are ignorant of the damage caused by RKN in their fields. This finding is in line with the study by Ijan*i et a*l. (2000) who reported that 80% of respondents in Morogoro were ignorant of the damage caused by RKN.

Moreover, this study has revealed that RKN are prevalent in all of the tomato fields surveyed in Mvomero District. This finding is in line with previous reports (Nono - Womdim *et al.,* 2002; , which reported RKN damage in different tomato growing areas in Tanzania. The widespread distribution of RKN in the study areas could be due to the prevailing favourable weather conditions and the polyphagous nature of RKN. It could also be attributed to continuous growing of susceptible tomato varieties on the same site and/or rotation of tomatoes with RKN susceptible crops such as okra and egg plant by the growers as noted during the survey. Santos *et a*l. (2019) reported the widespread of RKN associated with the cultivation of susceptible vegetable crops such as cabbage, pepper, carrot, eggplant, okra and tomato in Sub-Saharan Africa. Sei*d et al*. (2015) pointed out that tomato is a universal host for *Meloidogyne* spp.

2.7 Conclusions and Recommendations

This study has demonstrated that root-knot nematodes infect most of the cultivated tomato varieties in growers' fields in Mvomero District. However, the problem is neglected and considered as a low priority factor for crop production and protection. Majority of farmers are not aware that RKN is a serious tomato production constraint. Hence, there is a need for awareness campaign on how to diagnose and manage RKN in tomato growers' fields in Mvomero District.

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CHAPTER THREE

Paper Two

3.0 IDENTIFICATION OF ROOT - KNOT NEMATODES (*Meloidogyne* spp.) AFFECTING TOMATO IN MLALI, DOMA AND DAKAWA WARDS IN MVOMERO DISTRICT, MOROGORO REGION

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3.1 Abstract

Accurate identification of root knot nematode (RKN) is needed for appropriate management of this pest. Previous attempt to identify RKN in Tanzania were largely based on host range and morphological features that might be inconclusive due to overlap of perineal patterns between species. Therefore, this study was designed to identify species of RKN affecting tomatoes in farmers' fields in Mvomero district, using morphological descriptors and molecular (polymerase chain reaction [PCR]) techniques. Consequently, a survey was conducted in 2019 in five villages (Kipera and Mkuyuni) in Mlali, (Doma B and Kihondo) in Doma and Wami Dakawa in Dakawa wards of Mvomero District in Morogoro. Five tomato plants and rhizosphere soil samples per field (0.1 ha) were randomly collected from 15 small scale farmer fields about 25 cm deep using a hand shovel. Seventy-five tomato root and 75 rhizosphere soil samples were collected, packed (root and soil sample from each sampling point were combined in one

sterile plastic bag) loaded in cool box and transported to Tanzania Agricultural Research Institute (TARI) - Kibaha Nematology Laboratory. Single egg masses from collected root samples were individually used to establish 27 RKN single isolates populations for identification. Isolates were reared in RKN susceptible tomato cultivar Cal J in heat sterilised soil in the screenhouse at TARI – Kibaha. Examination of perineal pattern morphology was done under compound microscope and images were captured using a mounted camera. Molecular techniques targeting the mitochondrial DNA (mtDNA), internal transcribed spacer (ITS) region and SCAR primers were used to confirm the *Meloidgyne* spp. grouped according to the perineal patterns. The study revealed the presence of *Meloidogyne incognita* (18 populations), *M. javanica* (8 populations) and *M. arenaria* (1 population). The results on the diversity of *Meloidogyne* spp. affecting tomatoes in Mvomero District is useful in the development of strategic RKN management programmes.

Keywords: Identification, *Meloidogyne* spp., mitochondrial DNA, perineal patterns morphology, polymerase chain reaction.

3.2 Introduction

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are widespread, polyphagous endoparasites which cause economic loss in crops worldwide (. It is the hidden enemy of tomato because its participation remains ostensibly unclear due to their soilborne nature, minute size and concealed manner of life (Siddiqu*i et a*l., 2014). Therefore, accurate detection and quantification before planting are essential for sound pest management verdicts. Root-knot nematodes induce galls on the root system of a susceptible host which disrupt the vascular system. Their damage lead to symptoms such as reduced growth, chlorotic leaves, patchy growth, wilting, and premature plant death (Moens *et al.*, 2009;

Jones *et a*l., 2013). Increased host susceptibility to stress such as drought and other pathogens due to RKN infestation has been reported (Karssen *et al.,* 2013).

Several *Meloidogyne* spp. have been recorded worldwide (Hunt and Handoo, 2009), with *M. incognita*, *M. javanica*, *M. Arenaria*, *M. fallax* and *M. hapla* accounting for 95% occurrence of the genus (Ada*m et a*l., 2007). Morphological similarities existing between these species complicate their identification. However, distinguishing them is important for designing and implementing appropriate management strategies (Cunh*a et a*l., 2018). For examples, in order to develop rotation or resistant cultivars, accurate information on the nematode species present is crucial (Sikor*a et a*l., 2018). However, such information is scant in Mvomero District, which undermines the ability to make confident management recommendations.

Morogoro region, where Mvomero District is found, is one of the important tomato producing regions in Tanzania with the average yield of 9.5 t/ha (URT, 2017). Despite its production potential, Morogoro region is challenged by *Meloidogyne* spp. among other pests (Teste*n et a*l., 2018). However, little is known about the identity of *Meloidogyne* species affecting tomato in Mvomero District.

Meloidogyne spp. have been commonly identified based on morphology and morphometrics (Eisenbac*k et a*l., 1980). Chitwood (1949), used the perineal patterns morphology as a suitable diagnostic support and described *Meloidogyne hapla*. However, recent studies have revealed similarities between the perineal patterns of *Meloidogyne luci* and those of *M. incognita* (Carneir*o et a*l., 2004; Aydinli and Mennan, 2016). Despite this weakness, it is still used to discrete populations into species groups which is an important step towards identification of RKN (Mune*r a et a*l., 2010).

The chief molecular methods for the analysis of *Meloidogyne* species rely on the polymerase chain reaction (PCR), such as real-time PCR (qPCR), multiplex PCR, species-specific PCR and RFLP (Oliveir*a et a*l., 2011). Polymerase chain reaction methods based on DNA, are quick, more reliable, and are autonomous of the life stage of the nematode (Ada*m et a*l., 2007; Cunh*a et a*l., 2018). The use of molecular identification in species assay especially in *Meloidogyne* spp. helps to overcome complexes of cryptic species. With this laboratory technique, many copies of a specific DNA region are synthesised *in vitro*. It relies on a thermostable DNA polymerase (*Taq* polymerase) and requires DNA primers designed explicitly for the DNA region of interest. Species-specific primers can be designed and used to identifying *Meloidogyne* spp. (Zijlstr*a et a*l., 2000; Wishar*t et a*l., 2002; Corr*ea et a*l., 2014; Kiewnic*k et a*l., 2013; 2014). Nonetheless, a random selection of primers or combination of primers in a multiplex PCR is needed in order to determine the appropriate species-specific primer to use (Baido*o et a*l., 2016).

Previous studies on RKN in Tanzania were largely based on morphology and host range). This has led to limited available information on molecular characterisation of *Meloidogyne* spp. infesting tomatoes in the country. Therefore, this study was designed to identify of RKN to specie level in samples collected from tomato fields in Mvomero District by using morphological (the perineal patterns morphology) and molecular (PCR) techniques in order to suggest measures to manage RKN. This study formed a baseline for future studies that should focus on expanding surveys to map the distribution of *Meloidogyne* spp. in other tomato production areas of Tanzania that have not yet been surveyed.

3.3 Material and Methods

3.3.1 Description of the study area

The survey was conducted in October 2019 in farmers' tomato fields in Mlali (06° 57′ 0″ South, 37° 32' 0" East), Doma (7° 14' 0" South, 37°13' 0" East) and Dakawa (6° 26' 0"

South, 37° 42' 0" East) wards of Mvomero District in Morogoro Region, Tanzania. These wards are located at an altitude of 358 – 570 m above sea level. Laboratory investigations were undertaken at TARI Kibaha Nematology Laboratory (6° 46' 45.9" South, 38° 58' 24.0" East) while the establishment of pure culture of *Meloidogyne* spp. was done in screenhouse at TARI Kibaha (6° 46' 41.3" South, 38° 58' 20.6" East).

3.3.2 Soil and root sampling

A hand shovel was used to lift tomato plants and their roots to a depth of 25 cm as described by Coyne *et al*. (2018). Seventy five tomato root and 75 rhizosphere soil samples were collected randomly from 15 small scale tomato growers' fields in Mlali (5), Doma (5) and Dakawa (5) wards. Plot size for sampling was approximately 0.1 ha in each sampled farm. Root and rhizosphere soil from each sampling point were put together in one sample bag to represent a sample. Samples bags were clearly labelled, loaded in a cool box and transported to the TARI - Kibaha Nematology Laboratory where they were kept at 10°C until extraction. Coordinates from each tomato field surveyed were recorded using Global Positioning System (GPS) (Garmin-etrex 10, Taiwan).

Table 3.1: Areas *where sampling for plant parasitic nematodes was done*

Ward	Village	Field Code	Longitude	Latitude
Dakawa	Wami Dakawa	DA	037°32.270	06°26.657
		Dd	037°32.441	06°26.713
		Dh	037°33.951	06°24°.705
		Di	037°31.094	06°26.551
		Dj	037°31.094	06°27.729
Doma	Kihondo	DMa	037°16.841	07°06.590
		DMc	037°18.111	07°07.253
	Doma B	DMd	037°14.496	07°06.119
		DMe	037°14.457	07°06.160
		DMf	037°14.381	07°06.072
Mlali	Mkuyuni	Ma	037°31.576	06°57.818
		Mb	037°31.364	06°57.718
		Mc	037°31.446	06°57.889
	Kipera	Md	037°31.827	06°56.842
		Me	037°31.814	06°56.696

Source: This study

3.3.3 Establishment of pure cultures of *Meloidogyne* species

Tomato roots from the field were washed in running tap water to remove embedded soil and individually examined for the presence of egg masses under dissecting microscope (Leica MZ 95, Leica Microsystems, US). Egg masses of *Meloidogyne* spp. in affected tomato root system were randomly picked with fine sterilised forceps (Tay*e et* al., 2013). Each egg mass was transferred to 50µl of sterile water in single well in a multiple well tray. Eppendorf tubes containing egg mass were incubated at room temperature (25 - 28 °C) for 2 days. Freshly hatched juveniles (J2) from each single egg mass were inoculated to 3 weeks old seedlings of susceptible tomato cultivar Cal J (Pop Vriend (T) Ltd) raised in heat sterilised soil in 1 litre capacity pots. A micropipette was used to inject inoculum

via 3 holes, 3 cm deep made around the plant with a stick. Inoculated seedlings were maintained inside screen house at TARI - Kibaha.

3.3.4 Nematode identification

Nematode identification involved morphological approach which included examination of adult female perineal patterns and molecular approach.

3.3.4.1 Morphological identification

Perineal pattern studies were conducted using adult female of root knot nematodes from the first individuals of single egg mass cultures in order to minimise genetic variation (Aydinli and Mennan, 2016). Mature females were removed from selected Infected root pieces using forceps under dissecting microscope (Leica MZ 95, Leica Microsystems, US). Temporary slides were prepared by transferring ten randomly selected mature individual females from each population to slides containing a drop of water.

The head and neck regions of the nematode were excised and the posterior part placed in a solution of 45% lactic to remove the remaining internal contents. The observation of perineal patterns was done under compound microscope (Leica DM 2500, Leica Microsystems, US) by using a series of magnifications (10×, 20×, 40× and 100×), a drop of immersion oil was applied at 100× magnification. Images were captured by using a camera (GX CAM High Chrome - S, Version 8.5, GT Vision Ltd, UK) mounted on a compound microscope (Leica DM 2500, Leica Microsystems, US). Pictorial key from Hunt and Handoo (2009) was used to compare photos and key features for identification of each specie.

3.3.4.2 Molecular identification

This included DNA extraction from single specie populations, DNA amplification and evaluation of amplified products.

DNA extraction from single specie populations

DNA was extracted following the procedures described by Y*e et a*l. (2015). Individual mature females of *Meloidogyne* spp. from the same population were removed from selected infected root pieces using forceps under dissecting microscope (HUVITZ, HSZ-ZB700, GT Vision Ltd, UK). Each specimen was placed on a glass microscope slide (7.62 × 2.54 cm) in a 10 µl drop of TE buffer solution (10×; pH 8.0). Individual specimens were then ruptured with a yellow, flat-tipped micropipette tip (Nolato Treff, Switzerland), then added with 50-µl TE buffer. The lysate was pipetted into a 1.5 ml Eppendorf tubes and the tubes were stored in -20°C freezer.

DNA amplification

Primers pairs targeting the mitochondrial DNA and the internal transcribed spacer (ITS) and species-specific primers were used in the PCR reactions (Table 3.2). Amplification of DNA was performed in a volume of 25 µl reaction mixer containing 0.5 µl of each forward and reverse primers (10 µM) (Inqaba biotec) (Table 3.2), 2 µl template DNA, 9.5 µl of nuclease free water and 12.5 µl of one Taq Quick-Load 2× Master Mix (New England Biolabs, Ipswich, MA, US). PCR was conducted with a T-100 Thermal cylinder (Bio Rad Laboratories).

Amplification conditions consisted of an initial denaturation at 94°C for 4 minutes, 35 cycles of 95°C for 30 seconds, annealing at 50°C for 30 seconds and 68°C for 60 seconds, and final extension at 68°C for 7 minutes. PCR products were analysed using agarose gel

electrophoresis. During this procedure, 5 µl of each PCR product was mixed with 1µl of 10× loading dye (Glentham Life Sciences) and loaded on a 1× TAE (Tris – Acetate EDTA) buffer. DNA ladder (1kb plus, New England Biolabs) was added to estimate the sizes of the PCR products. After electrophoresis (220 V, 40 minutes) the gel was stained with SafeView™ Classic (Applied Biological Materials Inc., Richmond, BC, Canada) for 20 minutes, visualised and photographed under UVL.

Table 3.2*: Primers used for PCR*

Name	Region	Band size	Species	Reference
TRNAH/MORH106	mtDNA	557	Universal	Stanton *et* al. (1997)
rDNAitsF/R	ITS	400	Universal	this study
MtIngF/R	SCAR	560	*M. incognita*	this study
MtarF/R	SCAR	400	*M. arenaria*	this study
Mjar F/R	SCAR	300	*M. javanica*	this study

mtDNA = mitochondrial DNA, ITS = internal transcribed spacer region, SCAR = sequence characterised amplified region

3.4 Results

3.4.1 Establishment of pure culture

A total of 34 egg masses from RKN infested tomato roots collected from the fields in Mvomero District were used to set up pure cultures. Twenty-seven (27) out of the collected egg masses (34) were successfully cultured and ultimately used for *Meloidogyne* spp. identification.

3.4.2 Morphological identification of adult *Meloidogyne* spp.

Perineal patterns of three (3) *Meloidogyne* spp. were observed and pictured. Their images were compare with the pictorial key and features from Hunt and Handoo (2009). The patterns resembled with that of *M. incognita*, *M. arenaria* and *M. javanica*. *M. javanica* had rounded, to flattened dorsal arch and noticeable lateral lines separating the dorsal and

ventral regions of the patterns (Plate 3.1). *M. incognita* were characterised by a high and squarish dorsal arch (Plate 3.2). *M. arenaria* had ovoidal pattern with fine to coarse striae. Dorsal arch was low with smooth striae slightly wavy and slightly bent towards tail tip at lateral line; with shoulders on lateral portion of arch (Plate. 3.3). Dorsal and ventral striae met at an angle at lateral breeding lines.

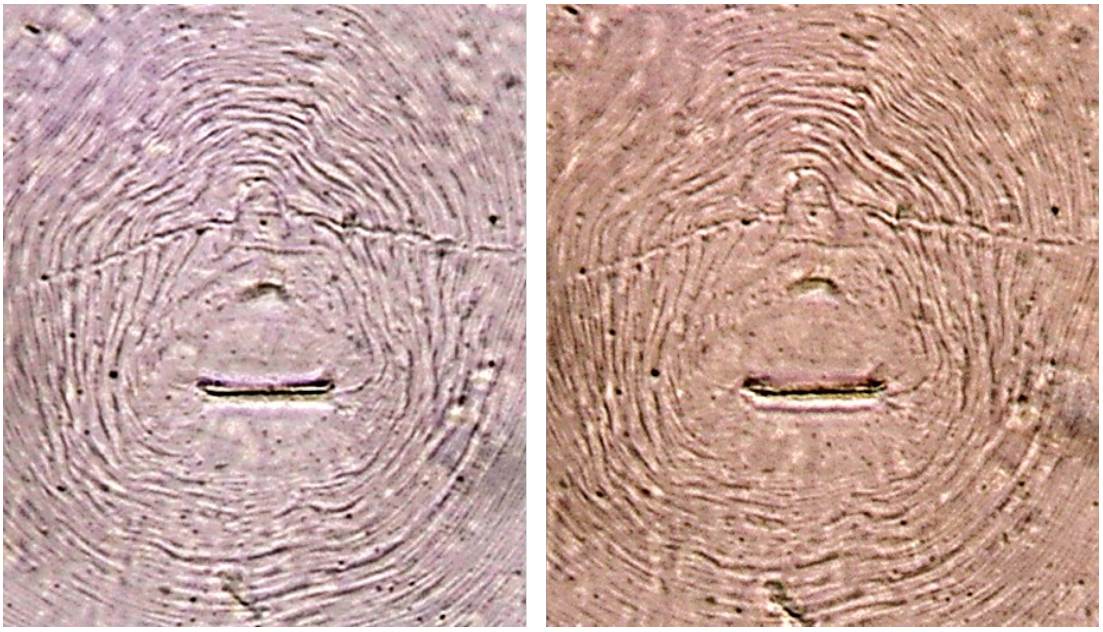

Plate 3.1: *M. javanica* compound microscope images (100× magnification) perineal patterns taken by Samweli Ombaeli at TARI – Kibaha nematology laboratory

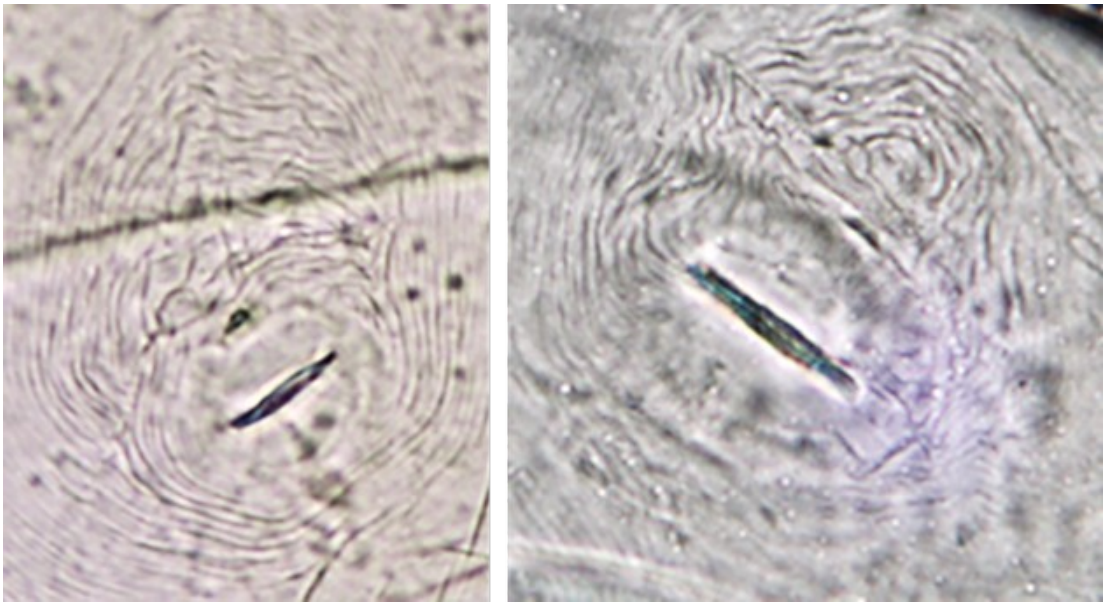

Plate 3.2: *M. incognita* compound microscope images (100× magnification) perineal patterns taken by Samweli Ombaeli at TARI - Kibaha nematology laboratory

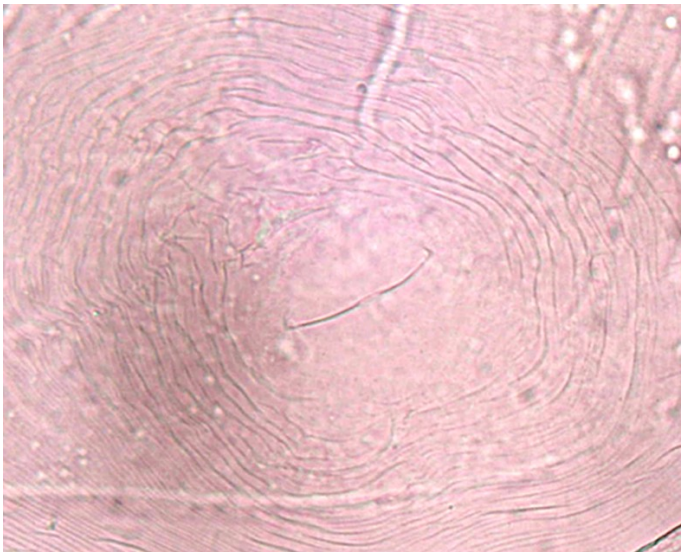

Plate 3.3: *M. arenaria* compound microscope image (100× magnification) perineal patterns taken by Samweli Ombaeli at TARI - Kibaha nematology laboratory

3.4.3 Molecular identification of *Meloidogyne* spp.

The primer pair TRNAH/MRH106 targeting the mtDNA yielded PCR products of 557 bp for the three tropical *Meloidogyne* spp. namely *M. javanica M. incognita*, and *M. arenaria*. Amplicons of 300 bp for the same *Meloidogyne* spp. were produced with primer set rDNAitsF/R targeting the internal transcribed spacer (ITS) region (Fig. 3.1). PCR with specific (SCAR) primer MtInF/R gave a positive band of 560 bp for *M. incognita.* Primer pairs MjarF/R and MtarF/R1 produced 300 bp and 400 bp products for *M. javanica* and *M. arenaria*, respectively in cultures (Fig. 3.1).

Meloidogyne spp. were observed to occur in a mixed species and single species populations. A mixture of *Meloidogyne incognita* and *M. javanica* was detected in three fields in Wami Dakawa, one fields in Kihondo and one field in Doma B villages. A mixture of *M. incognita*, *M. javanica* and *M. arenaria* was detected in Mkuyuni village. Single specie population of *M. incognita* was detected in one field in Kipera village (Table 3.3). Eighteen Out of 27 populations belonged to *M. incognita* while eight were *M. javanica* and one was *M. arenaria* (Table 3.3).

Table 3.3*: Root-knot nematode identification from single specie populations*

PCR code	Field coordinates		Location		Perineal patterns	Species specific PCR primers
	Longitude	Latitude	Ward	Village		
1	037°32.270	06°26.657	Dakawa	Wami Dakawa	Mi	MtInF/R1
2	037°32.270	06°26.657	Dakawa	Wami Dakawa	Mi	MtInF/R1
3	037°32.270	06°26.657	Dakawa	Wami Dakawa	Mj	MjarF/R
4	037°32.270	06°26.657	Dakawa	Wami Dakawa	Mi	MtInF/R1
5	037°32.270	06°26.657	Dakawa	Wami Dakawa	Mi	MtInF/R1
6	037°32.094	06°26.551	Dakawa	Wami Dakawa	Mi	MtInF/R1
7	037°32.094	06°26.551	Dakawa	Wami Dakawa	Mj	MjarF/R
8	037°32.094	06°26.551	Dakawa	Wami Dakawa	Mi	MtInF/R1
9	037°32.094	06°26.551	Dakawa	Wami Dakawa	Mj	MjarF/R
10	037°33.951	06°24.705	Dakawa	Wami Dakawa	Mi	MtInF/R1
11	037°33.951	06°24.705	Dakawa	Wami Dakawa	Mi	MtInF/R1
12	037°33.951	06°24.705	Dakawa	Wami Dakawa	Mi	MtInF/R1
13	037°33.951	06°24.705	Dakawa	Wami Dakawa	Mj	MjarF/R
14	037°33.951	06°24.705	Dakawa	Wami Dakawa	Mi	MtInF/R1
15	037°18.111	07°07.253	Doma	Kihondo	Mj	MjarF/R
16	037°18.111	07°07.253	Doma	Kihondo	Mi	MtInF/R1
17	037°18.111	07°07.253	Doma	Kihondo	Mi	MtInF/R1
18	037°18.111	07°07.253	Doma	Kihondo	Mi	MtInF/R1
19	037°14.457	07°06.160	Doma	Doma B	Mj	MjarF/R
20	037°14.457	07°06.160	Doma	Doma B	Mi	MtInF/R1
21	037°14.457	07°06.160	Doma	Doma B	Mi	MtInF/R1
22	037°31.364	06°57.718	Doma	Mkuyuni	Mj	MjarF/R
23	037°31.364	06°57.718	Mlali	Mkuyuni	Mi	MtInF/R1
24	037°31.446	06°57.889	Mlali	Mkuyuni	Mj	MjarF/R
25	037°31.446	06°57.889	Mlali	Mkuyuni	Ma	MtarF/R
26	037°31.827	06°56.842	Mlali	Kipera	Mi	MtInF/R1
27	037°31.827	06°56.842	Mlali	Kipera	Mi	MtInF/R1

Mi = *Meloidogyne incognita*, Mj = *Meloidogyne javanica*, Ma = *Meloidogyne arenaria*

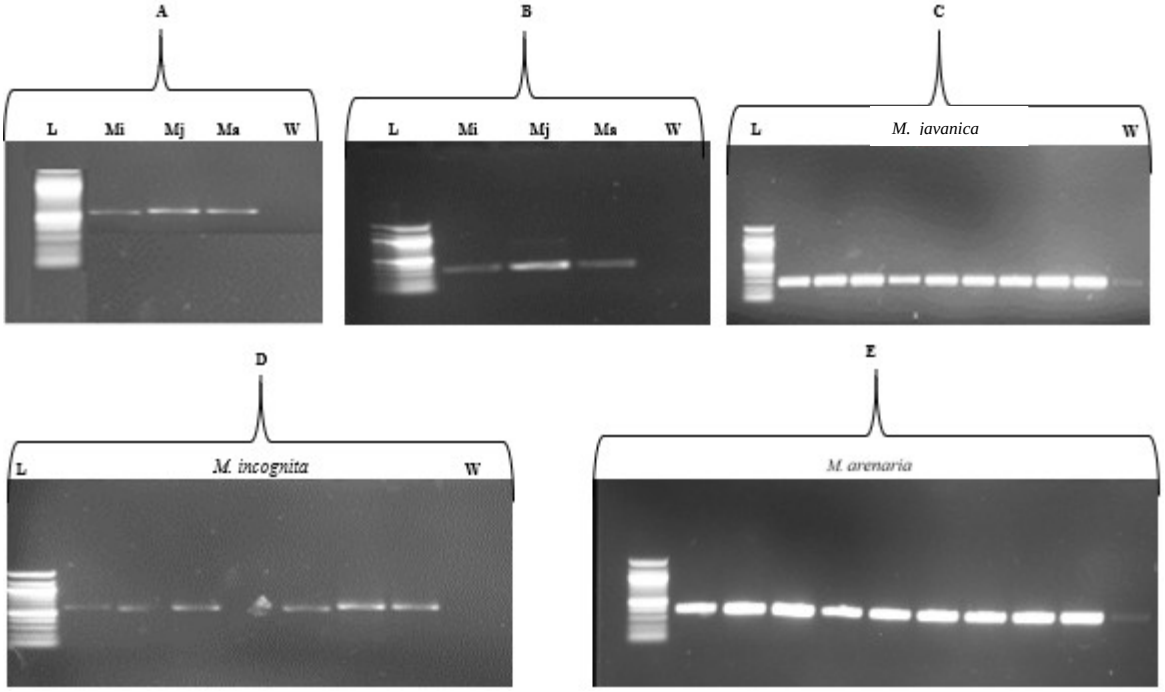

Figure 3.1: A & B = PCR products (557 & 300 bp) from mtDNA and internal transcribed spacer, respectively. Agarose showing sizes of amplification products from characterised *Meloidogyne* spp. obtained from primers TRNAH/MORH106 (A) and rDNAits F/R (B). Mi = *Meloidogyne incognita,* Mj = *M. javanica, M. arenaria,* L = 1kb plus DNA ladder with 100bp markers (New England Biolabs) and W = Non-DNA template (negative control). C, D and E = PCR products from mtDNA SCAR primers, Agarose showing sizes of amplification products from characterised *Meloidogyne javanica* 300 bp (C), *M. incognita* 560 bp (D) and *M. arenaria* 400 bp (E) obtained from SCAR primers Mjar F/R, MtIngF/R and MtaR F/R, respectively. W = Non-DNA template (Negative conrol)

3.5 Discussion

Meloidogyne spp. affect tomato production worldwide (Jones *et a*l., 2013), including Tanzania . The microscopic examination of the perineal patterns morphology of adult RKN females in pure cultures revealed the presence of three dissimilar species, *M. javanica*, M. *arenaria* and *M. incognita*. The perineal patterns were similar to those described by Hunt and Handoo (2009) and were confirmed by PCR.

The occurrence of *M. incognita*, *M. javanica*, and *M. arenaria* on tomato in Mvomero district suggests that tomato is a host to major *Meloidogyne* species. This could also be attributed to prevailing favourable weather condition and agricultural practices such as monoculture of the crop (Janat*i et a*l., 2018). The finding concurs with the study by Ijan*i et a*l. (2000) that reported prevalence of *M. incognita* and *M. javanica* in Morogoro Region. Furthermore, Nono-Womdim *et al.* (2002) reported the occurrence of *M. incognita* in Morogoro region.

The *Meloidogyne* spp. presented in this study are thermophilic (i.e. they thrive at relatively high temperature) with an ability to cause severe damage and generally distributed in tropical regions of the world (Hunt and Handoo, 2009). The dominance of *Meloidogyne incognita* over *M. javanica* and *M. arenaria* suggests that the former is adapt well to warm condition. *Meloidogyne javanica* and *M. incognita* have been reported as the most prevalent species of RKN occurring across Africa (Pagan *et al.*, 2015; Santo*s et a*l., 2019). Ijani *et al.* (2000) have also reported the predominance of *M. incognita* in the lowland areas of the Uluguru mountains is associated with hot dry conditions.

In some of the fields, *Meloidogyne* spp. occurred in mixed populations (Table 2.3). This could be associated with the ability of tropical species of *Meloidogyne* to cohabit. Similar

results were previously reported in Tanzania (Nono-Womdi*m et a*l., 2002), Uganda (Mwesege, 2013) and Pakistan (Anjum and Tariq, 2017). When using universal primer developed for *Meloidogyne* spp. (Stanto*n et al*., 1997), amplicon size of 557 bp were observed suggesting the presence of tropical *Meloidogyne* spp. (Fig. 3.6). The same primer set gave positive results in other studiesMoreover, species-specific primer developed for *M. javanica M. incognita* and *M. arenaria* gave characteristic bands of 300, 560 and 400 bp, respectively correlating with their morphological identification. This confirms the specificity of the primer sets. The use of species-specific primers was very helpful and gave confidence in the identification process. It served as a kind of supplementation and confirmation to perineal patterns result. Species specific primers for *Meloidogyne* spp. have successfully used in other studies (Zijlstr*a et a*l., 2000; Corr*ea et a*l., 2014; Kiewnic*k et a*l., 2014).

3.6 Conclusions and Recommendations

This study has used a combined morphological and molecular characterisation of *Meloidogyne* spp. from Mvomero district to avoid misidentification. The findings from this study such as prevalence of *M. incognita*, *M. javanica* and *M. arenaria* aids better understanding of the epidemiology of these important *Meloidogyne* spp. The results from this study will further provide a room for future research and for developing and implementing effective management strategies against identified RKN species.

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CHAPTER FOUR

Paper Three

4.0 THE REACTION OF TOMATO CULTIVARS AND BREEDING LINES TO ROOT- KNOT NEMATODE (*Meloidogyne incognita*.)

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4.1 Abstract

Fourteen tomato (*Solanum lycopersicum* L.) cultivars (Tanya, Kiboko, Tengeru 2010, Duluti, Meru, Rio Grande, Tengeru 97, Cal J, Zara F1, Assila F1, Jarrah RZ F1, Imara F1, Anna F1 and Kipato F1) and three tomato breeding lines (AVTO1424, AVTO1703 and AVTO1704) were tested for their response to *Meloidogyne incognita* in screenhouse pot experiment in 2020 at Tanzania Agricultural Research Institute – Kibaha. The experiment was organised in a Randomised Complete Block Design with four replications. Tomato cultivars/ breeding lines were treatments. Seeds were sown on peat moss filled seedling trays and transplanted singly into 4 litre plastic containers containing heat sterilised mixture of forest soil and farm yard manure (3:1, v/v) three weeks later. Seedlings were inoculated seven days after transplanting at the rate of 500 J2 per pot using 1 ml micropipette via 3 prepared holes, 3 cm deep. At the end of 12 week after inoculation, the experiment was terminated and data were recorded on fresh shoot and root weights and plant height. Root and soil populations of RKN were also determined. Collected data were

subjected to the analysis of variance using GenStat 20th Edition (VSN International, Hemel Hempstead, UK). Treatment means were compared using the least significant difference (LSD) at 5% significance level. A cultivar's/line's reaction to RKN was judged using root gall scores (GS) and reproductive factors (Rf). The cultivars tested differed significantly ($p < 0.001$) in gall scores (GS), Reproductive factors (Rf), shoot height, fresh root weight, fresh shoot and dry weights. Among the tomato cultivars and breeding lines screened against RKN, 11, 5 and 1 indicated susceptibility, resistance and tolerance, respectively. Identified RKN - resistant cultivars; Anna F1, Imara F1 and Assila F1, popular tomato cultivars in Tanzania, can be used to suppress RKN in infested fields to minimise yield loses caused by RKN.

Key words: *Meloidogyne* spp., resistance, susceptibility, tolerance, Tomato cultivars

4.2 Introduction

Tomato is produced in Tanzania by small-scale farmers and it contributes about 51% of total fruit and vegetable production (Mamir*o et a*l., 2015). The area under production in 2018 was 25 985 ha with a production of 356 094 tonnes (FAOSTAT, 2018). The crop is grown largely in Arusha, Iringa, Kilimanjaro, Mbeya, Morogoro, Mwanza and Tanga regions (Match Maker Associates, 2017). However, tomato is prone to attack by soilborne pathogens including root-knot nematode (Ramasamy and Ravishankar, 2018; Wanjoh*i et a*l., 2018). Root knot nematodes (*Meloidogyne* spp.) are obligate, soilborne, polyphagous group of plant parasitic nematodes (PPN) of global economic importance (Karss*en et a*l., 2013; Coyn*e et a*l., 2018). In some cases, they occur as a mixture in agronomic soils, each species with its exceptional host ranges and life-history characteristics (Powers, 2004). They induce galls of 1 – 2.5 cm on the root system of a susceptible host which disrupt the vascular system leading to symptoms such as reduced growth, chlorotic leaves, premature leaf abscission, wilting, decline in fruit production, premature death and increased

susceptibility to stress such as drought and other pathogens (Begu*m et a*l., 2012; Aydinl*i et a*l., 2013; Ralm*i et a*l., 2016). Symptoms are more widespread in tropical species compared to temperate root-knot nematodes (Sei*d et a*l., 2015). The magnitude of damage they cause in plants is exacerbated by their wide geographical distribution short life cycle (6 to 8 weeks) as well as a wide host range (Moen*s et a*l., 2009). Kokalis-Burelle and Rosskopf (2012), reported American jointvetch (*Aeschynomene americana*) and common purslane (*Portulaca oleracea*) as good hosts for *M. arenaria, M. incognita,* and *M. javanica.* making the development of sound management strategies complex (Gorn*y et a*l., 2019). Moreover, the level of damage generally depends on factors such as the nematode species, host plant, crop rotation regime, season and soil type (Moen*s et a*l., 2009; Olsen, 2011). The RKN can cause yield losses of about 30% by direct infestation and indirect losses due to predisposition or breakdown of resistance to other root ailments such as bacterial and fusarium wilts (Wanjiru, 2018).

Root-knot nematodes can be spread via infested plant material, agricultural tools, rain and irrigation water. They can also be spread through strong winds which carry infested soil particles and contaminated soil carried on shoes or animal feet (Muimba-Kankolongo, 2018). According to Coyn*e et a*l. (2018), *Meloidogyne* spp. are widely distributed across Sub Saharan Africa (SSA) attacking a wide range of cultivated crops. In Tanzania, *Meloidogyne* spp. namely *M. hapla, M. incognita, M. javanica* and *M. arenaria* inflicting tomato plants have been reported (Nono-Womdi*m et a*l., 2002).

Meloidogyne spp. have been managed with varying success by different physical, biological and chemical means. Chemical nematicides provide quick relief from nematode attack. However, the potential negative impact on environment and ineffectiveness after protracted use have led to ban or restricted use of these nematicides and an urgent need

for safe more effective alternatives (Zuckerman and Esnard, 1994). The modification of existing agricultural practices in order to manage RKN is one of the most acceptable alternatives to chemical control for both smallholder and large scale-farmers in the tropics (Sikor*a et a*l., 2018).

Tomato cultivars have varying degrees of susceptibility to *Meloidogyne* spp. with some cultivars being susceptible while others are tolerant or resistant (Singh and Khurma, 2007). The use of resistant tomato cultivars is one of the effective, safe and environmentally friendly strategies to inhibit reproduction of *Meloidogyne* spp. (Singh and Khurma, 2007; Cortad*a et a*l., 2009). Sorriba*s et al*. (2005) asserts that tomatoes carrying the *Mi-1 gene* are effective against *Meloidogyne* spp. and can be grown in nematode-infested soils without significant yield reduction. According to Roberts and Thomason (1989), *Mi-1* gene confers resistance to *Meloidogyne arenaria, M. incognita* and *M. javanica*. Resistant tomato cultivars inhibit pathogen reproduction better than susceptible ones leading to increased crop productivity (Cortad*a et a*l., 2009).

Some of the improved tomato cultivars available in Tanzania market are high yielding and resistant to disease such as fusarium wilt, early blight and tomato yellow leaf curl virus. However, their reaction to RKN is apparently unknown to small scale farmers. Identifying and using those cultivars with resistance to *Meloidogyne* spp. is crucial in enhancing tomato productivity in RKN hotspots for improved livelihood of tomato small-scale farmers.

This study was thus initiated to screen and identify resistance from against RKN in tomato cultivars grown in Tanzania.

4.3 Materials and Methods

4.3.1 Description of the study area

The Study was conducted in 2020 at Tanzania Agricultural Research Institute (TARI - Kibaha) screen house (6° 46' 41.3" South, 38° 58' 20.6" East) and Nematology Laboratory (6° 46' 45.9" South, 38° 58' 24.0" East). The area is located at an altitude of 125 m.a.s.l. with an average annual temperature of 25.5 ˚C.

4.3.2 Plant materials

Seeds of the tested tomato and breeding lines, AVTO1424 (CLN3682C), AVTO1703 (CLN3900C-23), AVTO1704 (CLN3900D) were obtained from the World Vegetable Centre (AVRDC) near Arusha, Tanzania. Open pollinated tomato varieties (OPVs): Tengeru 97, Tanya, Kiboko, Tengeru 2010, Duluti and Meru were also obtained from AVRDC near Arusha, Tanzania. Other open pollinated varieties (Rio Grande and Cal J); open field hybrids (Zara F1, Assila F1, Jarrah F1 and Imara F1); greenhouse hybrids (Anna F1 and Kipato F1) were sourced from local licensed Agro-input dealers in Morogoro. Varieties: Tengeru 97, Tanya, Kiboko, Tengeru 2010, Duluti and Meru were released in Tanzania and their seeds are affordable and available in the market. Rio Grande and Cal J are preferred because of their fruit colour, shape and good shelf life (Dhaliwal, 2001).

4.3.3 Raising of seedlings

Seeds were sown on peat moss filled in seedling trays. Three weeks old seedlings were transplanted singly into four (4) litre plastic containers containing heat sterilised mixture of forest soil and farm yard manure (3:1, v/v) (Tari*q et a*l., 2016).

4.3.4 Multiplication of RKN inoculum

The inoculum for screening tomato cultivars and breeding lines was prepared from established pure cultures of *Meloidogyne incognita* established and maintained in screenhouse at TARI-Kibaha. Root samples with RKN galling symptoms from all screenhouse maintained pots were gently washed in tap water to remove embedded soil and expose egg masses. Egg masses were handpicked individually using a disinfected forceps under compound microscope (Leica MZ 95, Leica Microsystems, US). Picked egg masses were place into a single well in multiple well trays containing 50 µl drops of sterile water and incubated at 25 - 28 ˚C. After 24 hours, the hatched-out juveniles were pipetted in a beaker containing sterile water. The volume of water in the nematode suspension was adjusted to 1 ml per 100 juveniles by decanting excess amount of water or by adding more water (Tari*q et a*l., 2016).

4.3.5 Inoculation of seedlings

Three holes three (3) cm deep were prepared three (3) cm from the base of four weeks old healthy tomato seedlings using a fine rod. The inoculum was agitated to ensure even distribution of inoculum before inoculation. The same was injected in three (3) holes at the rate of 500 J2 per pot using a micropipette. The experiment was organised in a Randomised Complete Block Design (RCBD) having 14 tomato cultivars and three tomato breeding lines (AVTO1704, AVTO 1424, and AVTO1703) as treatments in the screen house. Each treatment (cultivar/ tomato line) was replicated four times.

4.3.6 Agronomic practices

Plants were watered and fertilised with Yara Mila Winner (N.P.K 15:9:20) as needed while fungicide Ivory M-72 (8% Metalaxyl + 64% Mancozeb) and Selecron 720 EC (72% profenofos) were used as per the manufacturer recommendations to control fungal diseases and insect pest, respectively.

4.3.7 Data collection

Plant height, stem diameter and number of leaves per plant were recorded at the 12th week when the experiment was terminated. A tape measure was used to measure plant height from the root collar to the shoot tip. Individual plants were carefully uprooted at the end of the 12th week after inoculation and the shoot cut off at the soil line. The fresh shoot and root weight were taken before placing the shoots in the paper bags. Roots were gently washed in running tape water to remove embedded soil, visually observed for presence of galls and scored for galling of RKN using 1 (No galling) to 5 (severe galling) scale as illustrated by (Coyne and Ross (2014). The nematode reproductive factor (Rf) was calculated using the formula; (Rf = Pf/Pi), where Pi = initial inoculum level (500 juveniles) and Pf = final nematode population. For dry weight determination, plants were dried in paper bags in oven (Genlab Thermal Engineers, OV/150/SS/DIG, England) at 70°C for 3 days (Bozbug*a et a*l., 2020).

4.4 Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using GenStat for Windows 20th Edition (VSN International, Hemel Hempstead, UK). Where necessary, data on nematode counts were log transformed using the equation Log_{10} (x + 1), for normality. Treatment means were compared using Fisher's protected least significant difference (LSD) at p = 5%.

4.5 Results

4.5.1 Nematode infestation on plant growth parameters

The data presented in Table 4.1 unveil the response of different tomato cultivars and tomato breeding lines to *Meloidogyne* spp. Significant differences ($p < 0.001$) in number of leaves, shoot height and stem diameter were observed in nematode inoculated tomato cultivars and breeding lines. This indicates that tested tomato plants responded differently to RKN inoculation.

4.5.2 Effects of RKN on shoot and root fresh weights and shoot dry weights

Significant differences ($p < 0.001$) were noted in fresh root, fresh shoot and dry shoot weights among the tomato cultivars and tomato breeding lines (Table 4.1). Fresh and dry shoot weights, fresh root weight and plant height; decreased among inoculated tomato cultivars and tomato breeding lines compared with their respective controls (Fig. 4.1a, b, c, d). The shoot dry weights in all the inoculated tomato cultivars and breeding lines decreased as compared to the controls. The highest shoot dry weight reduction of 45% was recorded in cultivar Jarrah F1 while the lowest (1%) was recorded in cultivar Tengeru 97 (Table. 4.3).

Table 4.1: The effects of Meloidogyne spp. on growth parameters of tomato cultivars and tomato breeding lines inoculated with 500 J2

Cultivar	Shoot height (cm)	Fresh root weight (gm)	Fresh shoot weight (gm)	Dry shoot weight (gm)
Cal J	122.5a	75.12ab	232ab	26.5abc
Zara F1	135.2ab	68a	218ab	28.5abc
Tanya	139.2ab	64a	206.5ab	25.75ab
Jarrah F1	140.5ab	49.95a	188.8ab	33.75abc
Duluti	144ab	81.85ab	312.8ab	37abc
Imara F1	148abc	12.38a	185.2a	24.5ab
Kiboko	150abcd	57.18a	249.5ab	37.5abc
Assila F1	151.2abcd	50.62a	275.2ab	30.5abc
Tengeru 2010	155.5abcde	149.47b	322.5b	35.5abc
AVTO1424	157.2bcde	26.18a	237.8ab	40.25bc
Rio Grande	161bcde	76.47ab	215.5ab	23.5a
AVTO1704	161.5bcde	29.5a	202ab	27.25abc
Anna F1	178.8cdef	49.95a	201.8ab	31.75abc
Tengeru 97	179.8cdef	55.88a	312ab	39.75bc
Kipato F1	182.8def	70.95a	236.5ab	32abc
AVTO1703	186.8ef	47.75a	232.2ab	31.25abc
Meru	197.8f	66.95a	320b	42c
LSD (5%)	18.72	43.01	72.94	8.865
p-value	< 0.001	< 0.001	< 0.001	< 0.001

*Means within a column followed by the same letter are not different ($p \leq 0.05$); LSD = Least significant difference.

4.5.3 Root-knot nematode reproduction and gall development

There was significant difference ($p < 0.001$) in the recovered number of juveniles (J2) from 5 g of roots and those from 100 cm^3 of soil (Table 4.2). The mean RKN reproductive factor (Rf) among tomato cultivars and breeding lines varied significantly ($p < 0.001$). The lowest Rf of 0.2 was observed in Anna F1 and Assila F1 while the highest mean Rf of 4.7 was noted in Zara F1 (Table 4.2). Significant differences ($p < 0.001$) were noted for mean gall scores (GS) (Table 4.2). The result showed that out of the seventeen tested tomato plant materials, 11 were susceptible, one was tolerant and five were resistant. Anna F1, Assila F1, Imara F1 and AVTO1424 were found to be significantly resistant ($p < 0.001$) with GS < 3 and Rf < 1. Cal J, Rio Grande, Zara F1, Jarrah F1 and Kipato F1, AVTO1704, Duluti, Kiboko, Meru, Tanya and Tengeru 2010 were significantly susceptible ($p < 0.001$) (Table 4.2). However, the highest mean gall score of five (5) (GS = 5) was observed in Jarrah F1, Cal J, Kipato F1, Rio Grande and Zara F1. Tengeru 97 was significantly tolerant ($p < 0.001$) with mean GS of 3.00 and Rf of 1.1. AVTO1704 experienced RKN damage with mean GS and Rf of 4 and 1.9, respectively. AVTO1724 had mean GS and Rf of 3 and 0.8, respectively while AVTO1703 had GS and Rf of 3 and 1, respectively.

4.5.4 Correlation between variables

Significant ($p < 0.001$) linear positive correlation coefficients were observed between GS and Rf, fresh root weights and fresh shoot weight, GS and fresh root weight, dry shoot weights and fresh shoot weights (Table 4.3). Moreover, significant ($p < 0.05$) correlation coefficients were also determined between shoot height and dry shoot weight, reproductive factors and fresh root weights (Table 4.3). Shoot height and fresh shoot weights, gall scores and fresh shoot weight. The highest correlation coefficients were observed/ between fresh shoot weights and dry shoot weights (r = 0.73). Negative

correlations were also observed between gall scores and shoot heights, gall scores and dry shoot weights, Rf and shoot heights, fresh root weights and shoot heights, reproductive factor and fresh shoot weights (Table 4.3).

Table 4.2: The reaction of tomato varieties and tomato breeding lines inoculated with 500 J2 of Meloidogyne incognita per pot

Cultivar	Initial inoculum	Nematode count /100 g of soil	Nematode count/ 5 g of roots	Final nematode population	Mean gall score (1 - 5)	Reproductive factor (Pf/Pi)	Host's reaction
Cal J	500	445.5ab	1560.0d	2674 (3.43)cd	5.00c	4.0de	S
Zara F1	500	1084.2c	1253.0cd	3116 (3.49)d	5.00c	4.7e	S
Tanya	500	711.6bc	227.7a	2674 (3.43)abc	3.67abc	1.9abc	S
Jarrah F1	500	368.5ab	326.7ab	1047 (3.02)ab	5.00c	1.4ab	S
Duluti	500	74.5a	843.0abcd	1223 (3.09)abc	4.67bc	1.8abc	S
Imara F1	500	91.0a	116.7a	277 (2.44)a	2.67abc	0.4a	R
Kiboko	500	95.5a	900.0abcd	1327 (3.12)abc	4.33abc	2.0abc	S
Assila F1	500	57.2a	20.3a	103 (2.02)a	2.00ab	0.2a	R
Tengeru 2010	500	293.2ab	430.0abc	964 (2.98)ab	4.67bc	1.5ab	S
AVTO1424	500	57.5a	230.2a	417 (2.62)a	2.00ab	0.8ab	R
Rio Grande	500	711.0bc	542.7abc	1671 (3.22)abcd	5.00c	2.5bcd	S
AVTO1704	500	79.0a	859.5abcd	1251 (3.09)a	4.00abc	1.9abc	S
Anna F1	500	48.7a	44.0a	124 (2.39)a	1.67a	0.2a	R
Tengeru 97	500	73.7a	477.5abc	735 (2.97)a	3.00abc	1.1ab	T
Kipato F1	500	780.5bc	1130.0bcd	2547 (3.41)bcd	5.00c	3.8cde	S
AVTO1703	500	70.7a	336.0ab	503 (2.70)a	3.00abc	0.8ab	R
Meru	500	52.0a	553.7abc	808 (2.90)a	3.67abc	1.2ab	S
LSD (5%)		340.7	501.4	0.25	0.68	1.13	
p-value		<0.001	<0.001	<0.001	<0.001	<0.001	

Gall scores: 1 = no galls; 5 = all roots severely galled; R = resistance; S = susceptible; T = tolerance; Pf = final nematode population; Pi = initial nematode population. GS ≤ 3 and Rf ≤ 1 = R; GS ≤ 3 and Rf > 1 = T; GS > 3 and Rf > 1 = S. Numbers in parentheses are Log_{10} (x + 1) transformed means. Means within a column followed by the same letter are not different ($p \leq 0.05$); LSD = Least significant difference

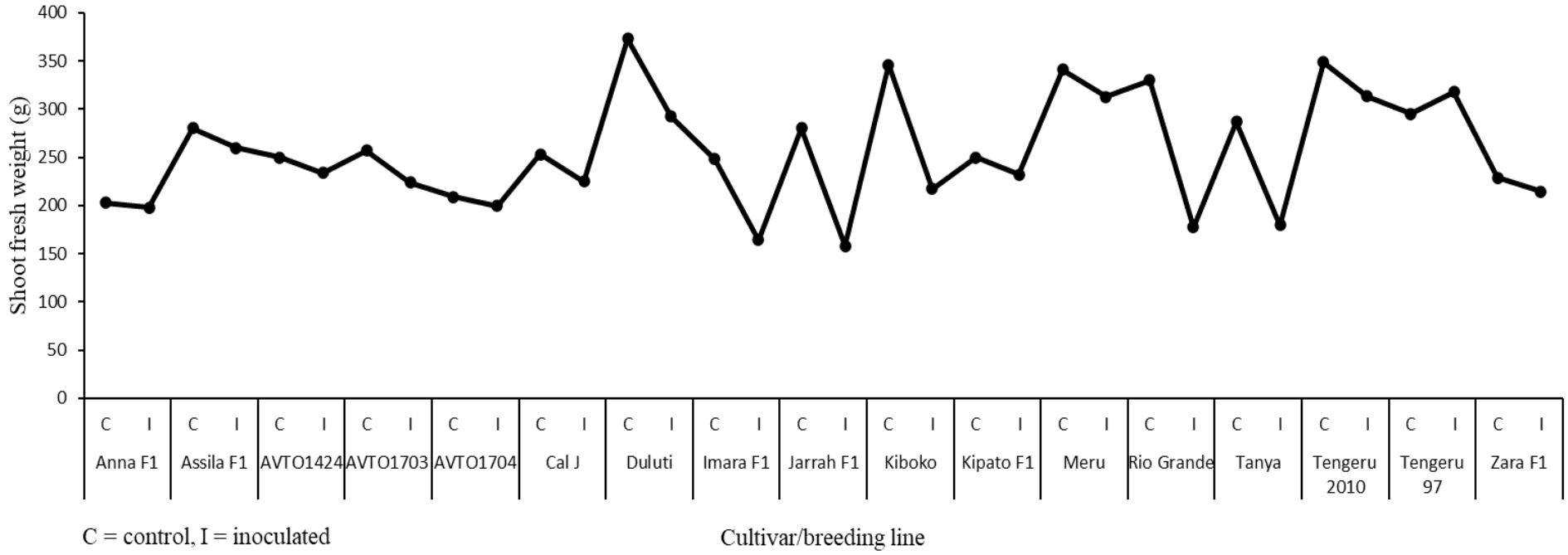

Figure 4.1a: Effects of RKN on fresh shoot weight of 14 tomato cultivars and three tomato breeding lines

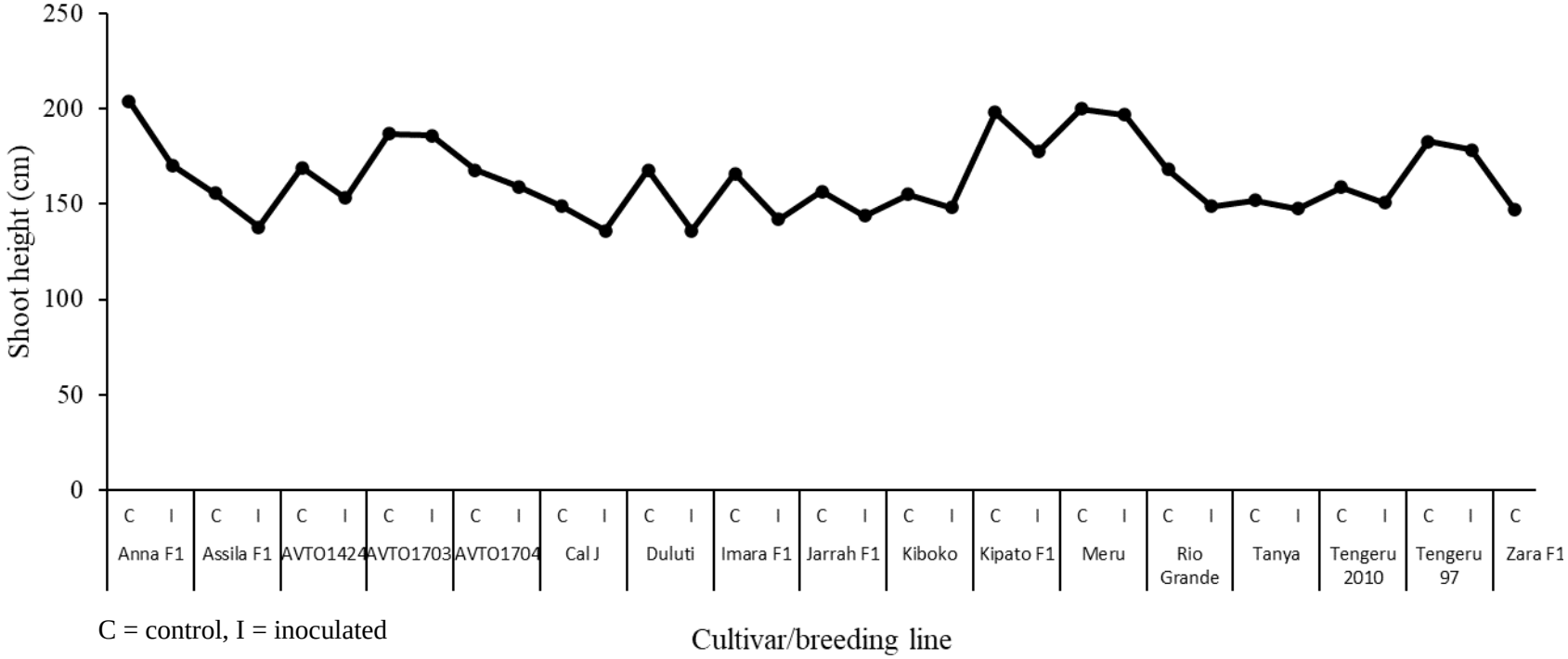

Figure 4.1b*:* Effect of RKN on shoot height of 14 tomato cultivars and three tomato breeding lines

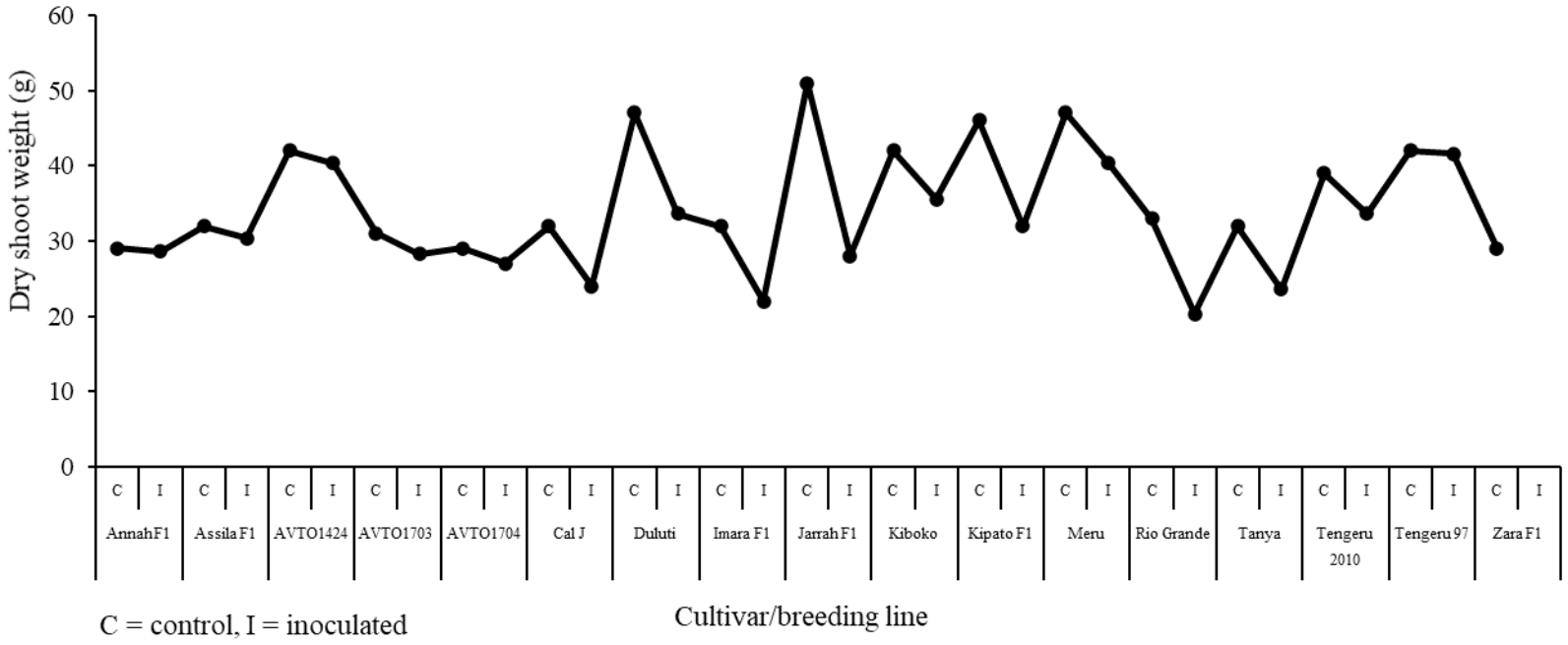

Figure 4.1c: Effect of RKN on dry shoot weight of 14 tomato cultivars and three tomato breeding lines.

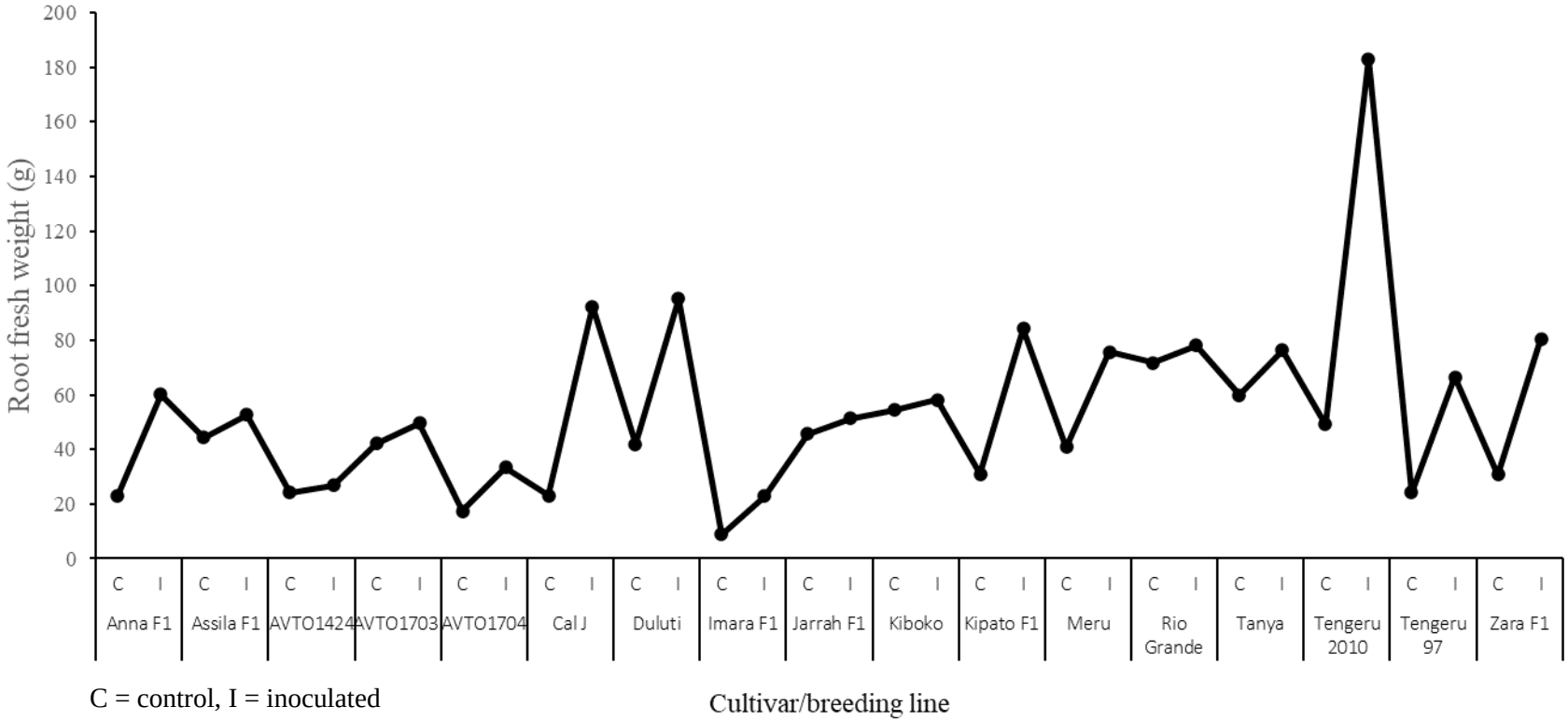

Figure 4.1d: Effect of RKN on fresh root weight of 14 tomato cultivars and three tomato breeding lines.

Table 4.3*: The reduction in shoot dry weight over control in tomato cultivars/line*

Cultivar/line	Inoculation	Shoot dry weight(g)	% reduction over control
Annah F1	C	29.0	
	I	28.6	1.4
Assila F1	C	32.0	
	I	30.4	4.7
AVTO1424	C	42.0	
	I	40.3	4.0
AVTO1703	C	31.0	
	I	28.3	8.7
AVTO1704	C	29.0	
	I	27.0	6.9
Cal J	C	32.0	
	I	24.0	25.0
Duluti	C	47.0	
	I	33.6	28.5
Imara F1	C	32.0	
	I	22.0	31.3
Jarrah F1	C	51.0	
	I	28.0	45.1
Kiboko	C	42.0	
	I	35.6	15.2
Kipato F1	C	46.0	
	I	32.0	30.4
Meru	C	47.0	
	I	40.3	14.3
Rio Grande	C	33.0	
	I	20.3	38.5
Tanya	C	32.0	
	I	23.6	26.3
Tengeru 2010	C	39.0	
	I	33.6	13.8
Tengeru 97	C	42.0	
	I	41.6	1.0
Zara F1	C	29.0	
	I	28.3	2.4

C = control; I = inoculated

Table 4.4: Pearson's correlation coefficients used to assess correlation among gall scores, reproductive factor, fresh shoot and root weights, dry shoot weights and shoot height on RKN inoculated tomato cultivars and tomato breeding lines and their related *p*-values

Variable	Dry shoot weight (g)	Fresh root weight (g)	Fresh shoot weight (g)	Gall scores (1-5)	Reproductive factor	Shoot height (cm)
Dry shoot weight (g)	1					
Fresh root weight (g)	0.2048	1				
	0.1494					
Fresh shoot weight (g)	0.7394	0.4573	1			
	<0.001	<0.001				
Galling scores	-0.1332	0.4537	0.0170	1		
	0.3514	<0.001	0.9058			
Reproductive factor	-0.0551	0.2774	-0.0165	0.4587	1	
	0.7007	0.0487	0.9083	<0.001		
Shoot height (cm)	0.4309	-0.048	0.389	-0.2184	-0.114	1
	0.0016	0.738	0.0048	0.1236	0.4257	

*Correlation coefficient values are significant at $p \leq 0.05$

4.6 Discussion

Significant differences in gall scores (GS) among the tested tomato plant materials showed different levels of response to inoculation. This variation could be ascribed to genetic differences associated with the presence/absence of resistance genes such as the *Mi* gene in the tomato cultivars/breeding lines tested. However, a successful RKN parasitic interaction could have exacerbated the modification of root parenchyma cells. Okorl*ey et a*l. (2018) reported significant differences ($p < 0.001$) in mean gall scores among *Solanum* rootstocks 12 weeks after RKN inoculation. Similarly, in the current study, severe galling was noted in susceptible cultivars, Jarrah F1, Cal J, Kipato F1, Rio Grande and Zara F1, 12 weeks after RKN inoculation. This could be linked to the high number of juveniles which penetrated the root and developed to maturity. Similar results were reported by Jiskan*i et a*l. (2012) in which susceptible tomato cultivar Roma was found to be penetrated by the greatest number of juveniles and produced the highest gall numbers.

In the previous study (Bagara*ma et a*l., 2014) observed the susceptibility of Tanya and Duluti to *Meloidogyne* spp., which is also evidenced in this study. Moreover, García and Sánchez-Puerta (2012) reported the susceptibility of the tomato cv. Rio Grande to *Meloidogyne* spp. Tomato breeding line AVTO1724 with *Mi* gene from AVRDC showed significant resistance ($p < 0.001$) to RKN parasitism. This could be credited to the ability of host resistance to impair *Meloidogyne incognita*. reproduction and root galling conferred by *Mi* gene within these breeding lines. Bozbug*a et a*l. (2020) reported that the *Mi* resistance gene is mostly specific leading to various expression levels to be noticed during the reaction of host resistance. Tengeru 97 which is among the improved tomato cultivars released in Tanzania, exhibited tolerance to *Meloidogyne incognita*. Similarly,

Minj*a et al*. (2013) documented tolerance response of the aforementioned cultivar to RKN.

On the other hand, low mean GS was observed in resistant cultivars, Anna F1, Assila F1, Imara F1 and the tomato breeding lines AVTO1424 and ATO1403. The resistance of Assila F1 to populations of *Meloidogyne incognita* and *M. javanica* has also been reported by Sei*d et a*l. (2017). This could be resulted from the hypersensitive reaction triggered by resistant cultivars which impaired the ability of juveniles to penetrate the roots and establish feeding sites. Low gall scores resulting from RKN inoculation has been reported in resistant hosts (Mwesege, 2013; Kau*r et* al., 2014; Okorl*ey et* al., 2018).

The significant decrease in shoot height over controls in the inoculated tomato plants as observed in this study could be the evidence that RKN infestation might have caused a lagging behind in shoot growth. *Meloidogyne* spp. damage has been reported to impair the normal growth of the plants, resulting into stunted growth (Muimba-Kankolongo, 2018). Dry shoot weight reduction caused by nematode infection in addition to gall scores (GS) and reproductive factor (Rf) are important in evaluating plant reaction to *Meloidogyne* spp. (Husain, 1986). Dry shoot weight reduction over controls varied significantly ($p < 0.001$) among the tested tomato plant materials. However, susceptible cultivars inoculated with *Meloidogyne* spp. showed greater percentage decrease in dry shoot weight than the resistant ones. This could be attributed to *Meloidogyne incognita* damage in roots of susceptible cultivars which impaired minerals uptake from the soil and negatively affect photosynthesis resulting to reduced dry matter accumulation. These results concur with that of Aalder*s et a*l. (2009) who reported the significant ($p < 0.001$) decrease in shoot dry weights over control in *Meloidogyne* spp. inoculated tomato cultivars. Moreover, Mwang*i et* al. (2017) reported greater reduction in dry shoot weight

in RKN inoculated tomato cultivar Cal J. Comparable trend has been reported to occur in other crops such as fenugreek (*Trigonella foenum-graecum*) (Tari*q et a*l., 2016), okra (*Abelmoschus esculentus* L.) (Hussai*n et a*l., 2014) and carrot (*Daucas carota*) (Siddiqu*i et* al., 2016).

There was a significant ($p < 0.001$) increase in root fresh weights corresponding with a decrease in shoot fresh weights in susceptible cultivars. The reason for this could be due to the establishment of feeding sites in roots by RKN which might have attracted plant photosynthate accumulation in roots in favour of RKN growth. Similar results were reported by Fortnu*m et a*l. (1991) who observed a significant ($p < 0.001$) increase in fresh root weight matching with the decrease in fresh shoot weight in plants infected with *Meloidogyne incognita*. The author further reported that *M. incognita* causes reduction in fresh weights with variation in shoot tissues of plant biomass. This has been revealed in this study whereby shoot heights and dry weights varied significantly ($p < 0.001$), which could either be associated to soil nutrients in the growing medium or RKN infestation which led to poor plant growth (Bozbug*a et a*l., 2020). The reduction in plant weight compared to the control in RKN inoculated tomato cultivars was also reported by Singh and Khurma (2007).

4.7 Conclusions and Recommendations

Determining the presence of resistant and susceptible/tolerant tomato cultivars is critically important to control RKN. The results from the current study have significant agronomic implication as they demonstrate the availability of resistance to *Meloidogyne incognita*. resistance in some tomato cultivars currently available in the market. Cultivars Anna F1, Assila F1 and Imara F1 may be utilised as an economically and environmentally feasible option for managing RKN populations in farmers' fields.

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CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. The study has revealed that 59% of respondent tomato growers in Mvomero District were not aware of RKN problem.

ii. The study also has found out that RKN prevailed in all of the three wards surveyed i.e., Mlali, Doma and Dakawa

iii. The study has further confirmed the existence of three *Meloidogyne* spp. i.e. *M.incognita*, *M.arenaria* and *M. javanica* in Mvomero District.

iv. The dominant RKN specie in all the study areas was *Meloidogyne incognita*.

v. *Meloidogyne arenaria* was only detected in Mlali.

vi. Cultivars Anna F1, Assila F1, Imara F1 and tomato breeding lines AVTO1424 and AVTO1703 showed significant resistance to *Meloidogyne incognita*.

5.2 Recommendations

i. There is a need for awareness campaign on how to diagnose and manage RKN in tomato growers' fields in Mvomero District.

ii. Farmers in Mvomero District should be encouraged to grow tomato cultivars Anna F1, Assila F1, Imara F1 which revealed resistance to *Meloidogyne* spp.

iii. The results on *Meloidogyne* spp. diversity in tomato fields in Mvomero District should be used for future research and in developing effective RKN management strategies.

iv. Screening of new tomato cultivars against *Meloidogyne* spp. should be done on a regular basis so as to provide a wide range of choices to tomato growers.

v. The results from this study are based on screen house conditions, there is therefore, a need to conduct more studies under field conditions in naturally *Meloidogyne* spp. infested soils.

vi. There is a need for conducting further studies which will include bigger sample size for a better conclusion.

vii. Extensive assessment of RKN is needed in order to enable a better understanding of *Meloidogyne* spp. occurrence, distribution and host preferences in other parts of Tanzania where tomato is grown under intensive production.

APPENDICES

Appendix 1: Questionnaire

Root-knot nematodes and other pests inflicting tomato in Mgeta and Mlali Wards and their management.

Enumerator: Introduce yourself and explain the purpose of this survey, which is to collect information on the prevalence root knot nematode affecting tomato, current control measures, knowledge on root knot nematode on tomato and its management. Please explain that the information solicited is for research purposes only. Remember there are no wrong answers to the questions.

1. Basic data

Date form filled (dd/mm/yyyy) ……

District ……Ward ……Village…….

Hamlet ……...

2. Respondent's personal data

a) Respondent's name ……..

b) Sex of respondent: 1. Female……... 2. Male …….....

c) Age of respondent.....years

d) Highest level of education of respondent (tick one): (i) None …… (ii) Primary ……. (iii) Secondary (Form iv) ……. (iv) Secondary (Form vi) …… (v) Technical/college/certificate/diploma ……. (vi) University ….....

Form (v) and (vi) please indicate specialisation e.g. Certificate in Agricultural General.

Specialisation if (any).....

3. Knowledge on tomato production:

a) How long have you being producing tomato? ……

b) How much land do you grow tomato? ……..

c) How much do you harvest per acre? ……..

d) Mention tomato varieties you are growing

ii) …… ii) ……. v) ……

iii) …… iv) ……. Vi) ……..

e) Where do you obtain seeds?

i) From agro-dealers …….

ii) Own saved ……

f) Do you normally exchange tomato seedlings with your neighbours?

(Yes//No).....

g) Do you rotate tomato with other vegetables? (Yes//No)..... After how many seasons……

Mention the vegetables that you include during rotation

i) ……. iii) …… iii)..……

ii) ……. iv) ……. v) …….

4. Knowledge on tomato pests including root knot nematode (diseases and weeds):

a) List the names of the four (4) most important diseases, and weeds that damage your tomatoes (*ask if there is a local name for each of the pests mentioned*)

Diseases
1.
2.
3.
4.
Weeds
1.
2.
3.
4.

b) Of the pests listed above, rank the most destructive pest:

Pests ranking (Start with the most destructive)
1.
2.
3.
4.
5.
6.
7.
8.

c) How do you control root knot nematodes of tomato in your field?

……
……
……
……

d) Do you use pesticides to control pests in tomato? 1. Yes..... 2. No ……

e) If the answer for the above question (4d) is **"yes",** fill in the Table below:

Name of pesticide	**Name of target pest**	**Application frequency per season**

f) If the answer for the question number 4d is **"no",** give reason why

……
……
……
……
……

g) Does root nematode occur in your field?

1. Yes …….. 2. No……..

h) Is there any botanicals or non-chemical pesticides you can use to manage root knot nematode in tomato? 1. No…….. 2. Yes ……

i) If the answer for the question number 4 (h) is **"yes"** mention them
……
……
……
…….

5. Knowledge on pesticides handling (tick one in each row)

Activity	**Always**	**Sometimes**	**Never**
(1) Do you read labels on the pesticides container before using?			
(2) Do you wear protective clothing and other accessories like nasal mask, eye goggles and boots when applying the pesticides?			
(3) Do you mix pesticides with your hands?			
(4) Do you observe the pre-harvest waiting periods after applying the pesticides?			
(5) After spraying, do you wait 12 hours before entering the field?			
(6) Do you store pesticides in a secure, sound and well-ventilated location?			
(7) Do you make a cocktail before applying the pesticides (*i.e. mix more than one chemical and apply them at once*)?			

a) Do you store pesticides? 1. No ……. 2. Yes …….

b) Where do you store your pesticides?
……
c) Why do you store the pesticides?
……

Thank you!

Appendix 2: Root-knot nematodes galling index scale (Coyne *et al.*, 2014)

0 = no galls

1 = 1 – 2

2 = 3 – 10

3 = 11 – 30

4 = 31 – 100

5 = More than 100 galls