EVALUATION OF THE GROWTH AND YIELD OF SOLANUM LYPopersicum L.
PECTOMECH GRAFTS IN ROOTKNOT NEMATODE INFESTED SOILS

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL CROP
SCIENCE DEGREE

DEPARTMENT OF CROP SCIENCE

COLLEGE OF BASIC AND APPLIED SCIENCE

UNIVERSITY OF GHANA, LEGON.

JULY, 2016
DECLARATION

I, CHARLES AGYEMAN, do hereby declare that except for references cited which have been duly acknowledged this thesis “Evaluation of the growth and yield of Solanum lycopersicum L. ‘Pectomech’ grafts in Root knot Nematode Infested Soils” is the result of my own research. It has never been presented either in part or in whole for the award of any degree.

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DEDICATION

I dedicate this thesis to the Almighty God, creator of Heaven and Earth and his only begotten son the Lord Jesus Christ who died on the cross that all might be saved and have everlasting life.
ACKNOWLEDGEMENT

My deepest gratitude goes to Dr. Naalamle Amissah and Dr. S.T Nyaku; my supervisors, whose guidance, suggestions, and involvement saw to the completion of this work.

My deepest appreciation goes to my mother Elizabeth Adjei for her love and financial support which saw me throughout my period of study.

My heartfelt appreciation goes out to my friend Maudline Oye Okyere for her encouragement, support, advice and prayers.

This research thesis is part of the Office of Research Innovation and Development (ORID), University of Ghana funded project titled “Evaluation of vegetative growth, yield and fruit quality of grafted tomato plants in root-knot nematode infested soils”. I am grateful for their financial support through the principal investigator Dr. Naalamle Amissah of the Crop Science Department, College of Basic and Applied Sciences without which this project would not have been possible.
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ABSTRACT

This study evaluated the resistance, agronomic performance, yield, fruit quality and shelf life of *Solanum lycopersicum* “Pectomech” grafted (cleft method) onto four *Solanum* spp. rootstocks (*Solanum macrocarpon*, *Solanum aethiopicum*, *Solanum lycopersicum* “Mongal”, and *Solanum lycopersicum* “Samrudhi”) under four root knot nematode densities (0, 500, 1000 and 5000). It consisted of a greenhouse pot experiment, field pot experiment, and open field experiment. The experimental design used was a 4x4, 2x3 factorial experiment in a split plot design and a randomized complete block design with three replicates respectively. The interaction effect between the *Solanum* rootstocks and the nematode densities had no significant effect on gall score index and the nematode reproductive factor (GI ≤ 2, RF ≤ 1). However, screening of the rootstocks using Mi gene specific primers (PMIR3/F3 and C2D1/C2S4) revealed that the rootstocks *Solanum lycopersicum* “Samrudhi and *Solanum lycopersicum* “Mongal possessed the Mi gene with double PCR band sizes of 300 bp and 500 bp and a single band size of 800 bp for the primer pair of PMIR3/F3 and C2D1/C2S4 respectively whilst *Solanum macrocarpon* and *Solanum aethiopicum* bands did not possess the Mi gene. The grafting success of Pectomech grafted onto *Solanum macrocarpon* and *aethiopicum* on the average were higher (93% and 94%) compared to the grafting success of *Solanum lycopersicum* “Mongal (0%). There was no significant interaction between rootstocks and the nematode densities on, fruit quality and shelf life of tomatoes, however, in the open field experiment the grafted rootstock effect significantly increased the yield in Pectomech grafted onto *Solanum aethiopicum* (461g/plant) compared to the un-grafted Pectomech (152 g/plant). The grafted rootstocks also offered resistance to the spread of *Fusarium oxysporum f.s. lycoperici* which is host specific to tomato compared to the non-grafted Pectomech an indication that grafting has multiple functions in crop protection.
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family and is believed to have originated from Southern and Central America. However, it was not until the 16th century that the crop was introduced by the Portuguese to Ghana (Amuti, 1971). Tomato is one of the most lucrative and widely cultivated crops of the Solanaceae family worldwide because of its high rate of consumption in various countries around the world (Johannes *et al.*, 2000).

In Ghana, the tomato fruit is prominent in various local or traditional dishes where it is used in the preparation of soups or stews. Tomato can also be consumed fresh as a vegetable or processed into various forms such as canned tomato paste or sauce. Its beneficial antioxidant properties have been shown to protect the consumer against health related diseases such as cancer (Di Mascio *et al.*, 1998). Tomato production in Ghana is mainly centred in six of the ten regions namely Ashanti region (Akumadan and Ejura), Volta region, Brong-Ahafo region (Wenchi, Tanoso, Derma), Central region (Mfantiman East), Upper East and West regions, with the total land area under cultivation estimated at 28,400 ha and 37,000 ha in 1996 and 2000 respectively (GIPC, 2001).

Tomato cultivation in these areas has by far become an indispensable crop to both farmers and non-farmers (traders) alike serving as a major source of employment and income for their livelihood and the national economy as a whole. In spite of the many benefits that the country enjoys from this crop (job creation, income generation and foreign exchange), its production
faces numerous pest and diseases challenges leading to low yields and a huge tomato deficit driven by an increasingly high demand for the fruits of this crop (Wolff, 2009).

Some of the reasons why more and more farmers are experiencing low yields in relation to the cultivation of this crop have to do with the damage of the crop by root knot nematodes (*Meloidogyne* spp.), *Fusarium* spp., bacterial wilt and fungal diseases (Simpson and Sarr, 2001). The nature of the damage caused by these microscopic organisms has been described as parasitic in nature, Root knot nematodes for instance feed on nutrients absorbed by the plant’s roots depriving it of essential nutrients and water, in effect the root weight of the plant increases reducing shoot growth of the plant thus resulting in the wilting of the plants and eventually death in extreme cases (Roberts, 1995).

In an attempt to control the economic damage caused by disease-causing organisms in vegetable production in Ghana, several measures have been recommended which includes the application of nematicide and organo-synthetic fungicides such as methyl bromide and the planting of resistant varieties (Sherf and MacNab,1986). However, in recent times, many of these chemicals and fumigants have been withdrawn from the market because of their harmful effects on the environment. In addition, farmers incur additional cost in procuring these agro-chemicals which can significantly decrease their profit margins.

One technique that holds promise in controlling pest and plant diseases in vegetable production is vegetable grafting. Grafting refers to the joining of tissues of two different plant species of the same family to form a single plant (Hartmann *et al.*, 2002). In grafting, a scion and a rootstock are joined together to form a complete plant, the scion refers to the shoot system or the upper part of the plant which produces fruits and the root stock basically refers to the root system or the lower part of the plant (Mudge *et al.*, 2009).
In other major tomato producing and exporting countries like Japan and the USA, farmers have resorted to grafting desired cultivars onto specific rootstocks, which has been proven to be resistant to soil-borne diseases and nematodes and also a substitute for the use of chemicals to disinfect soils. Over 40 million grafted tomato seedlings are being used in North American greenhouses to improve pathogen resistance using identified rootstocks (Kubota et al., 2008). Therefore, a research into the use of the grafting technique will help improve the yield of tomatoes grown in potential pathogen infested soils.

1.2 Justification

Tomato cultivation is one of the major lucrative economic activities in Ghana especially among farmers in the three Northern regions (Upper East, Upper West and Northern region). In the Upper West region for instance, the crop is estimated to be more profitable than the cultivation of rice and groundnut together (Jaiteh, 2010).

According to GIPC, tomato earned the country 25,000 US dollars in 2001(GIPC, 2001). Despite the economic importance of this crop to the Ghanaian, farmers who grow this crop face numerous challenges with various pests and diseases such as root knot nematodes, fungal, viral and bacterial infections respectively with most varieties being susceptible to these diseases as well as root knot nematode infections (Horna et al., 2006). The root knot nematode (Meloidogyne spp.) is the most destructive form of pathogens serving as a carrier for most of the fungal, viral and bacterial diseases which affect the growth and yield of tomato.

This research, therefore, seeks to evaluate the effect of grafting Pectomech, the most widely cultivated tomato variety in Ghana onto two Solanum rootstocks. (Solanum macrocarpon and
Solanum aethiopicum) noted for their tolerance to root knot nematodes and plant disease-causing pathogens for root knot nematode tolerance, agronomic performance, yield, and fruit quality attributes.

The specific objective of this experiment were to assess the:

- Level of resistance of Solanum macrocarpon and Solanum aethiopicum root-stocks to root knot nematode infestation in a greenhouse pot experiment.

- Agronomic performance (yield, shelf life and fruit quality attributes) of grafted tomato plants in varying density of Meloidogyne spp. inoculated soils in open field pot experiment.

- Agronomic performance (yield, shelf life and fruit quality attributes) of grafted and non-grafted tomato plants in root knot nematode infested open field.
CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 Origin and botany of tomato

Tomato (Solanum lycopersicum) is a juicy berry fruit which belongs to the plant Solanaceae family also called nightshade, tomato is a native of South America and is said to have arrived in West Africa through trade with the Portuguese (Tindal, 1983). It is a climbing annual which grows to a height of 2m or more depending on the prevailing environmental conditions and agronomic practices. The stem and leaves are hairy in nature with the leaves spirally arranged in an alternating manner along the stem. Generally, the majority of tomato plants produce compound leaves as well as flowers which are borne in a cluster of the inflorescence of 4-12 flowers with a diameter of 1cm (Williams, 1991).

The tomato plant often develops roots along its stem by producing small bumps also known as root initiators which enlarges the root system and the potential health of the plant, the root system of tomato makes up one-third of the fresh weight of the plant which features a large tap root system and a network of small roots (www.mobot.org). The roots serve as an anchor, absorbing and transporting water and nutrients to other parts of the plant as well as storing sugars and proteins for the manufacture of fruits. The fruits exist in many shapes and colors depending on the variety and breed, when the fruits are unripe they have an all-around green color, however, upon ripping they may be red, yellow or even orange in color and kidney or pear-shaped (Tindal, 1983).

Tomato fruits are classified as a berry and it develops from an ovary after pollination and fertilization of the plant has taken place, the fruits that are formed have hollow spaces which
contain seeds and depending on the variety a fruit may have two to five hollow spaces called locular cavities. The seeds in a mature tomato fruit can be extracted and nursed for propagation after drying it for a period of time (Jacobsen et al., 1994).

2.2 Cultivation of tomato in Ghana

The cultivation of tomato in Ghana is an important economic activity which engages over 90,000 farmers, 5,000 traders and about 3,000 individuals who play a role in the tomato value chain (Robinson and Kolavalli, 2010). Most of these economic activities are centered in the Northern, Upper East and the Southern Volta regions of Ghana. In some of these areas the cultivation of tomatoes has been found to be more profitable than other food crops such as rice, maize and groundnut and more than 90% of the people who live in these areas are said to be actively involved in the cultivation of the crop (Jaiteh, 2010).

The common tomato varieties in major tomato producing towns such as Akumadan, Sege, Dodowa, Wenchi, Agogo and Ejura, are Roma, Pectomech, Burkina, Royal and Power (Khor, 2006). Till date, yield of tomato per hectare basis is very low with an average output of fewer than 10 tons per hectare under rain fed conditions (Robinson and Kolavalli, 2010). This can be attributed to several factors such as the sensitivity and vulnerability of the plant to various diseases including fungal, viral, bacteria and root knot nematode infections as well as high post-harvest losses (Horna et al., 2006).

Most farmers also cultivate a particular tomato variety on the same piece of land yearly without any crop rotation practices which encourage the rapid multiplication of soil pathogens, across the country tomato farmers are experiencing low yields because most farmers do not adhere to strict crop husbandry management regimes, for instance most farmers still broadcast their seeds
creating unfavorable competition among seedlings for light, nutrients and continuously cultivating the same land which has led to the prevalence of soil borne pathogens. In an effort to mitigate against this problem farmers resort to the use of agrochemicals which most of them abuse posing health risks to consumers (Ellis et al., 1998).

2.2.1 Pests and Diseases of tomato

The major challenge that farmers face in cultivating tomato in Ghana has to do with the damage of the crop by pathogens, these pathogens (bacteria, fungi, viruses and root-knot nematodes) cause various diseases which include tobacco mosaic, Fusarium wilt, and blight. Some common insect pests which are associated with the cultivation of tomato include aphids, whiteflies, cutworms and flea beetles (Freedman et al., 2008).

The white flies which transmit the leaf curl virus disease are small white-winged insects which easily fly off with little disturbance, they hide beneath the leaves of the tomato plant for shelter and in the process they suck the sap of the leaves whilst transmitting the virus. Unlike the tomato leaf curl disease, the tomato mosaic virus disease is transmitted through mechanical injuries, and it comprises of several related viruses which cause tomato leaves to mottle and wilt prematurely (Obeng-Ofori et al., 2007).

2.2.2 Root-knot nematode (Meloidogyne spp.)

Nematodes compared to other soil microbes are the most abundant in soils worldwide. They are cosmopolitan causing yield losses of up to about 17-20% annually which is estimated at 100 billion dollars (Bird et al., 2003). There exit about 60 different plant-parasitic nematode species a figure which represents 19 genera of those which attack tomato, about 95% of the species identified do feed on roots either within the root tissues or outside the root tissues as endo or
ecto-parasites respectively. However, more than 90 species have been identified to be sedentary endo-parasites (Wesemael et al., 2011). Sedentary endo-parasites (Meloidogyne spp.) are considered to be one of the most successful parasites occurring in different climatic and geographical areas, they are capable of interacting with different plant pathogens to cause damage to plants thereby reducing plant yield.

The root-knot nematode (Meloidogyne spp.) belongs to the order Tylenchida, Suborder, Tylenchina, Superfamily, Hoplolaimoidea, Family, Meloidogynidae, and Genus Meloidogyne. It is the most destructive nematode on horticultural and field crops (Norman, 1992). They are obligate parasites hence capable of feeding inside the roots of plants and they penetrate the roots of the plant by making an opening in the root using a stylet and migrate intercellularly through the root. Whilst in the plant they form specialized feeding cells in the root (giant cells and galls) and redirect plant nutrients and other photosynthates to meet their energy demands (Hunt et al., 1991). They also interfere with water and nutrient uptake by the plant causing nutrient deficiency symptoms such as yellowing, wilting, loss of vigor, stunting and the decay of plant tissues due to secondary infection and a reduction in yield (Perry et al., 2009).

From an economic perspective, root-knot nematodes are ranked among the top three most damaging genera plant parasitic nematodes with the females being more destructive than the males. In Ghana yield losses of tomato attributed to the damage caused by root-knot nematodes in the Guinea savannah zone in the Northern region was once estimated to be around 73 to 100% (Hemeng, 1981). Farmers who are able to identify the secondary infection of the root knot nematode have to procure nematicides to manage the damage caused which increases the cost of production, an additional cost which will be passed on to consumers (Moens et al., 2009).
2.2.2.1 Biology and life cycle of root-knot nematodes (*Meloidogyne* spp.).

The name *Meloidogyne* which is synonymous with root-knot nematodes is said to be of Greek origin which means “Apple-shaped female.” Exactly 100 species of these apple-shaped female nematodes have been identified with *M. incognita*, *M. javanica*, and *M. arenaria* being the widest spread in the tropics. A typical characteristic of most plant parasitic nematodes is the possessions of a stylet, an organ which the nematode uses to pierce its host to enable it to inject certain secretions which dissolve plant cells and allows it to ingest nutrients from the host plant. They also lack a skeletal framework and possess an outer covering (cuticle) which acts against the detrimental effect of internal turgor pressure and also maintain the shape of the organism as well as aiding their movement through the soil micelle (http://www.apsnet.org).

Among this species (*Meloidogyne* spp.) the female is the most destructive and hence causes great damage to plants on the field, they can be identified under a microscope based on their globose shape which ranges from 400 to 1000µm in length and a short neck which has other organs such as the oesophageal gland cells, metacarpus and a piercing organ (stylet). However, one common and efficient way of identifying the female is through the use of perineal pattern analysis. This method is based on the fact that the region surrounding the vulva and anus displays a consistent pattern of ridges and annulation which is synonymous with only the female root knot nematode (Jepson, 1987).

Plant parasitic nematodes generally have a relatively simple life cycle which consists of the egg, four juvenile stages and an adult stage (male or female) which coincides with four molts. The root-knot nematode (*Meloidogyne* spp.) which is a sedentary endo-parasite completes most of its life cycle within its host (Mai and Abawi, 1987), with such a relatively short life cycle which
lasts between six to eight weeks, the root-knot nematodes are able to multiply at a faster rate ensuring their survival within a suitable host (Shurtleff and Averre, 2000).

In the absence of a suitable host, root knot nematodes survive in the soil as eggs and juveniles. In the first stage, juveniles develop in the egg and undergo molting giving rise to a second stage juvenile which emerges or frees itself from the eggshell into the soil mass. After hatching from the egg, the first stage juvenile moves through the soil mass to find a susceptible plant to infect or feed on, once the nematode finds a susceptible host it penetrates the host root tip region feeding on the tissue of the plant root while at the same time undergoing a second, third and fourth molt into a male or female.

The root cells which are fed on in the vascular tissue enlarge and develop into galls which provide nutrients to the root knot nematode and also serve as a place of abode. Upon copulation and fertilization, the female produces about 300 to 800 eggs into a gelatinous matrix or egg mass which protects the eggs from unfavorable environmental conditions. Most often the egg mass of the root-knot nematode is embedded in the tissue of the root of the plant (Mai and Abawi, 1987)

2.2.2.3 Symptoms of root-knot nematode (Meloidogyne spp.) infection.

Symptoms of root-knot nematode infection in tomato production are often confused with nutrient deficiency symptoms due to some similarities. Most farmers are unable to tell when their plants have been attacked by these pathogens until their yields are heavily affected. During the process of infecting the roots of the plant, the conducting tissues get damaged which predisposes the plant roots to bacteria and fungi in the soil and in the process nutrients are leached into the soil (Sasser, 1989).
The severity and appearance of symptoms depends on the species and density of nematodes present, the susceptibility of the crop to those nematodes and the environmental conditions under which the plants are cultivated. The symptoms associated with root-knot nematode infection can be grouped into above ground and below ground symptoms, the above-ground symptoms which occur on the shoot systems of the plant is mostly not noticed at the onset of the infection by the pathogens, but as the infection rate (in the form of galling of the roots) increases the above ground symptoms become visible leading to the appearance of chlorosis of the leaves with purple discoloration of the leaves, stunted growth of the plant as well as the wilting or browning of the plant in the presence of nutrients, moisture and other favorable conditions (Ekanayake, 1993). The below-ground symptoms include the galling of roots, reduction in weight and size of the root system. The severity of galling on roots is normally an indicator of the population or inoculum load of nematodes in the soil.

Increasing inoculum load of *Meloidogyne* spp. increases the infection rate of the plant by the pathogen which also reduces the yield and growth characteristics of infected plants. Different inoculum levels do affect tomato genotypes differently as the inoculum loads increases from a lower level to a higher one, as the levels of inoculum are increased the number of nematodes that penetrate and gain entry into the roots and tissues of the plant also increase causing more damage to the plant irrespective of the root-knot nematode species involved (Maleita *et al.*, 2012). Also increasing nematode inoculum levels does reduce plant height, stem girth and fresh and dry root weights. (Kankam and Adomako, 2014).

In another research by Bawa *et al.* (2014), on the pathogenicity study of southern root-knot nematodes (*Meloidogyne incognita chitwood*) on Roma king tomato cultivar with three inoculum levels of (6,000, 8,000 and 10,000 eggs), flowering, fruit yield, the number of leaves and plant
height all experienced significant reduction. The fruit weight of the tomato plants which were inoculated with the highest inoculum level (10,000 eggs) also recorded a 100% loss in fruit weight, on the other hand, chlorotic leaves and root galling weights all increased significantly.

In evaluating the response of some genotypes of tomato to the root-knot nematode specie *Meloidogyne incognita* in a pot experiment, Khanzada *et al.* (2012), reported that when the five tomato cultivars (Gola France, Roma v.f, Anmol, Roma and Sunehra) were inoculated with a second stage larva of *M. incognita* for two months, the agronomic parameters such as plant height, root weight and shoot weight, as well as total growth, were significantly reduced compared to the un-inoculated plants. This demonstrates that not only does nematode infection reduce yields but also other growth parameters such as the shoot weight and root weights are equally affected.

### 2.2.2.4 Molecular Identification of root-knot nematode species.

Traditionally differences in the morphological characteristics of microorganisms (plant parasitic nematodes) has been the sure way of differentiating between different organisms belonging to a family or species. This method of identifying microorganism requires personnel who have expert and detail knowledge of the morphological traits of the organism in question (Berry *et al.*, 2008). However, because individual infectious pathogens or microorganisms differ from each other based on their DNA as well as variations in their DNA code at the protein level (Cunningham and Meghen, 2001), they can be identified individually using nucleic acid sequencing even when their DNA are mixed up in a complex biological sample or materials such as plant tissues.

The use of molecular techniques such as PCR, (polymerase chain reaction) with primers that are specific to a particular plant parasitic nematode are used to identify or amplify sections of the
DNA of a plant parasitic nematode that is peculiar to that particular nematode. The use of this technique increases the sensitivity of distinguishing between a number of nematode species using a highly conserved morphological trait (Blok, 2005).

For instance, *M. incognita, M. arenaria, M. javanica* and *M. hapla* can be sensitively distinguished using PCR-restriction fragment length polymorphism (RFLP), on the other hand, *M. incognita* and *M. javanica* can be diagnosed using real-time PCR. In recent times, other techniques which involve the use of PCR sequenced characterized amplified region (SCAR), analysis of mitochondria DNA (mtDNA), ribosomal DNA (rDNA) and PCR amplification using species-specific primers have all been used to identify *Meloidogyne* spp.

The identification of nematodes using PCR is based on the size of the specific PCR product (bands) and the absence or presence of the PCR products when specific primers are used in a gel electrophoresis. In an experiment conducted by Berry *et al.* (2008) to detect and quantify three nematode species (*M. javinica, P. zeae* and *X. elongatum*) of sugarcane production in South Africa using SYBR green dye 1 and real-time PCR technology, two primers which were specific to root-knot nematodes with sequences Melo R long (5’-3’) GGCCTCACTTAAGGCTCA and Melo R short (5’-3’) TATACAGCCACGGACGTTCA were used in the identification of the individual nematodes. Each of the two reverse primers (18S+ Melo R long and 18S+ Melo R short) which were used to amplify and quantify the PCR product of *M. javanica* gave a discernible band size of 380 bp and 300 bp respectively.

In another experiment conducted by Lewis *et al.* (2001), to identify species of root-knot nematode *M. incognita, M. hapla, M. javanica* and *M. arenaria*, DNA were extracted from 26 different single egg mass nematode isolates and used to identify species-specific sequenced
tagged sites, species-specific primers with sequence pairs were developed and used to identify specific species.

*(M. arenaria* 5’-TCGAGGCGCATCTAATAAAGG-3’ 5’-GGGCTGAATATTCAAGGAA-3’
*M. hapla* 5’-GGCTGAGCATAGTAGATGATGTT-3’ 5’-ACCCATTAAGAGGAGTTTTGC-3’
*M. incognita* 5’-TAGGCAAGTAGTTGTCGGG-3’ 5’- CAGATATCTCTGCAATTGTC-3’
*M. javanica* 5’-CCTTAATGTAACACTAGAGGC-3’ 5’-GGCCCTAACCAGACATTAGA-3’).

The various species-specific primer pairs that were used, amplified and produced unique PCR product (bands), single bands of 1,500 bp, 950 bp, 1,650 bp were produced for *M. hapla*, *M. arenaria* and *M. javanica* respectively with a double band of 1,350 bp been recorded for *M. incognita*, however, when the four primer pairs were combined and used for a multiplex assay PCR bands were not formed or produced.

Contrary to the findings of Lewis *et al.* (2001), where a multiplex PCR assays did not produce bands, an experiment carried out by Hu *et al.* (1992) a multiplex PCR was used to identify and detect three *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M. enterolobii*) whose DNA were extracted directly from individual galls produced bands.

One pair of *Meloidogyne* universal primers (MF 5’-GGGGATGTTTGAGGCAGATTG-3’ MR 5’-AACCCTTCGGACTTCCACCAG-3’) were designed based on the conserved regions across the *Meloidogyne* nematode in the 28S rRNA D2D3 expansion domain and three pairs of the primers,

(Mi-F 5’-GTGAGGATTACGCTCCCAG-3’ Mi-R5’-ACGAGGAACCTTTACTCTGCCAG-3’
Fjav 5’-GGTGAGCCGATTGAACCTGAGC3’ Rjav 5’-CAGGCGCCTTCAGTGGCAACTATAC-3’
Me-F 5’-AACTTTTGTGAAATGTTGCGCTG3’ Me-R 5’-TCAGTTCAGGCAGATCAACC-3’).
3’) for *M. incognita*, *M. javanica* and *M. enterolobii* respectively were used. The multiplex PCR product from the species generated two fragments 500 and 1000 bp, 500 and 200 bp and 500 and 700 bp for *M. incognita*, *M. enterolobii* and *M. javanica* respectively.

### 2.2.2.5 Management of root knot nematodes.

The infection and damage caused by root-knot nematodes mostly occur in the production of vegetables with an average economic yield loss of 10%. However, higher yield losses of 30% have been recorded in the production of susceptible crops such as tomatoes and melons. Such high yield losses have led to the development of various management systems which can be grouped into non-chemical and chemical management practices (Sikora and Fernandez, 2005). The chemical management practices involves the use of nematicides such as methyl bromide, however, environmental concerns over the negative impact of these chemicals on the environment have led to a ban on its usage and in some jurisdiction its use is restricted (Collange *et al.*, 2011).

Other non-chemical management practices (cultural practices) such as solarisation, land rotation and bush fallow, ploughing of infested fields during the dry season, vegetable grafting, crop rotation, use of resistant cultivars, flooding, incorporation of organic amendments into the soil, burning of stubble after harvest, sanitation, fertilization and the planting of antagonist plants such as marigold (*Targetes erecta*) as an intercrop are also employed. In trying to put in place management systems to minimize the damage caused by these pathogens, farmers must choose integrated management practices which will be cost effective and not increase their cost of production at the expense of profits and the sustainability of their farms.
Individual management practices may have its own challenges depending on the geographical location and the prevailing weather conditions. For instance, flooding of the soil reduces the density of *Meloidogyne incognita* by displacing all oxygen molecules in the soil to create an anaerobic condition (Collange et al., 2011). This condition suppresses the multiplication of the nematode and requires flooding of the field for 8 weeks, a requirement which may be difficult to meet especially among vegetable farmers who practice rain-fed farming. Other management practices such as land rotation and bush fallow cannot be implemented in areas where there is a scarcity of agricultural lands, breeding of resistant crop varieties may also lead to increases in seed prices which may raise affordability issues.

2.3 Fusarium wilt

Fusarium wilt is mainly caused by a fungus called *Fusarium oxysporum*, which consists of several collections of fungi that are distinct in terms of their morphology and attacks several food crops such as tomatoes, sweet potato, banana and leguminous plants in general (Aktions-Netzwerk, 2010). *Fusarium oxysporum* is classified into divisions called formae speciales of which there are about 80 divisions based on the plants that they attack (host specific) with varying symptoms such as wilt and crown rot.

The fungi *Fusarium oxysporum* f. sp. *Lycopersici* is wildly noted to be host specific to tomato even though this fungus is soil borne it can equally live in plant tissues such as the xylem tissue of tomato (Grattidge and O’Brien, 1982).

2.3.1 Biology and life cycle

Fusarium wilt mostly occurs in areas where the climate is warm with soils mostly being acidic, the fungi *Fusarium oxysporum* f. sp. *Lycopersici* can, therefore, survive in such soils as
Chlamydospores for years in wait for an alternative host (Wong, 2003). They can also live in the remains of dead plants whilst making use of micronutrients in the soil as well as root exudates of plants growing in the soil.

Whilst in the soil the fungus invades plants through natural openings in the root systems of plants as well as openings created by root knot nematode infestation favored by cool temperatures and wet or waterlogged soils (Paulus, 1991). Generally, *Fusarium oxysporum* f. sp. *Lycopersici* plated on a PDA exhibits a subtle whitish to pinkish growth with a purple trace, it also grows three asexual spores ranging from micro conidia to with three to five septate as well as chlamydospores.

### 2.3.2 Symptoms of Fusarium wilt infection in tomatoes

Symptoms of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Lycopersici* can sometimes be confused with bacterial wilt caused by *Ralsotnia solanacearum* since both diseases occur and begin with yellowing of the leaves. However, the first observed indication of the disease is the yellowing of the lower or older leaves which coincides with fruit maturity (Roberts *et al.*, 2001). Affected plants generally wilt during the day and tend to recover during the latter time of the day which gradually leads to the browning of the leaves and eventually wilting. A cross-section of the roots and stem of infected roots also shows the browning of the xylem tissues or the vascular tissues with brown streaks along the length of the roots or stem (Wong, 2003).

### 2.3.3 Control/Management of Fusarium wilt in tomatoes

Like other pathogenic diseases finding a permanent cure for Fusarium wilt is very difficult and can be close to impossible. Several chemical and non-chemical management systems have been recommended to effectively manage this disease with some level of success, according to Ioannou (1999) soil solarization is one of the effective means of managing cases of fusarium wilt.
outbreaks in tomato production. This technique can reduce the population density of the pathogen (*Fusarium oxysporum* f. sp. *Lycopersici*) in the soil.

Presently the use of synthetic agrochemicals such as carbendazim and Mannitol which serves as pesticides is also used as a means of controlling fusarium wilt but there are also concerns over their effects on the environment due to their low biodegradability (Priyanka *et al.*, 2010).

In view of the various constraints that come with the implementation of some of these management regimes vegetable grafting is seen as a possible alternative due to its environmental friendliness coupled with its ability to control pathogens and diseases such as root knot nematodes and Fusarium wilt respectively.

### 2.4 Vegetable grafting

Grafting refers to the natural or deliberate joining or fusion of plant parts (scion and rootstock) of different plants which are of the same family (taxonomy) such that it results in a composite plant. The top portion which is the scion is normally chosen to harness certain desirable attributes such as high yields per unit area, size of fruits, nutrition, and flavor whilst rootstock are chosen for their disease resistance attributes to pathogens as well as their ability to grow vigorously to enable the uptake of nutrients and water (Lee and Oda, 2003). The use of grafting as a technique in vegetable production originated from Japan and Korea where it was used to effectively control yield losses resulting from infections by soil-borne diseases and pathogens especially against nematodes (Lee *et al.*, 2010).

#### 2.4.1 Importance of vegetable grafting

Vegetable grafting serves as an effective means of controlling soil-borne pathogens and diseases, and increasing fruit size and crop yields. In advanced countries such as the U.S and Holland
where Greenhouses and tunnels are used to cultivate vegetables, vegetable grafting is used to reduce a number of agro-chemicals (fumigants) used to control soil-borne diseases because of the restriction in practicing crop rotation under Greenhouse conditions (Oda, 1999). Vegetable grafting has also been observed to increase fruit yields of vegetables such tomato and eggplants (Lee and Oda, 2003). It has also been reported that grafting enhances the uptake of nutrients as well as improve water use efficiency (Colla et al., 2010).

An improve water use efficiency and nutrient uptake enables grafted plants to withstand short dry spells as well as increase photosynthetic activity respectively. Under flooding conditions grafted plants have also been noticed to have survived under water for several days when eggplant rootstocks were used (Black et al., 2003). Even though vegetable grafting has been shown to increase fruit yields some contrary reports suggesting that vegetable grafting is more deleterious than advantageous in terms of the quality of fruits that are produced by grafted plants (Proiertti et al., 2008).

2.4.2 Selection of rootstock for tomato grafting

The reason behind grafting a particular crop variety on to another crop variety is dependent on the genetic attributes of the two crop varieties involved, most often farmers choose or select rootstocks which have some desirable genetic attributes such as resistance to nematode, flooding, salinity, high or extreme temperature levels and the ability to increase yields. Commercially, apart from crops belonging to the cucurbitaceous family (melon), tomato and eggplants are the most grafted plants of the Solanaceous family (King et al., 2010).

The most common rootstocks used for commercial tomato grafting are hybrids (F1) or interspecific hybrids which have been specifically bred for resistance against pathogens and other diseases such as nematodes, Vetricillum wilt and Fusarium wilt. Hybrids are produced by
crossing selected tomato varieties with other wild *Solanum* species which have the genetic ability to offer resistance to specific diseases and pathogen attack or infection (Verdejo-Lucas *et al.*, 2013).

In some parts of Europe for instance tomato hybrids are preferably used as rootstocks than any other *Solanum* spp., because of their high level of genetic improvement compared to the other *Solanum* species (King *et al.*, 2010). However, other plants which share the same family with tomato (*Solanum torvum, Solanum aethiopicum* and *Solanum macrocarpon*) can also be selected and used as rootstocks for their tolerance to waterlogged and drought conditions, Fusarium wilt and root knot nematode infestation (Black *et al.*, 2003).

An ideal rootstock for tomato grafting should not only be one that is resistant to pathogens but also be compatible with the scion of the tomato and as well as express a high level of vigor and resistance to pest and diseases. A vigorous rootstock is expected to confer that level of vigorousness to the scion. On the other hand, a root stock that exhibits a very high level of vigorousness relative to the scion can also be a disadvantage to the scion were the tomato graft will grow more vegetative at the expense of fruit yield and quality (Yamakawa, 1982).

In developing countries the use of tomato hybrids as rootstocks may not be popular because of the cost involved in purchasing imported hybrid seeds, the use of eggplants as rootstocks remains the best alternative to hybrids with *Solanum torvum, and Solanum macrocarpon* and *Solanum aethiopicum* (Kumba group) being the most promising in terms of its usage as a rootstock against pathogens and to increase yield even though large-scale grafting using *S. torvum* is not viable due to its low or poor germination of its seeds (Gisbert *et al.*, 2011). On the other hand, there are conflicting claims which suggest that eggplants are not entirely resistant to pathogens. According
to Tzortzakakis et al. (2006), an evaluation of accessions of ten *Solanum* species (*Solanum. aethiopicum*, *Solanum. macrocarpon*, *Solanum. torvum*, *Solanum. gilo*, *Solanum. violaceum*, *Solanum. melongena*, *Solanum. incanum*, *Solanum. linnaeanum*, *Solanum. coccineum* and *Solanum. dasyclinum*) against toboavirus and root knot nematodes showed no resistant to root knot nematode infection, hence none of the tested *Solanum* accession had the potential to be used as resistant root-stocks for the grafting of tomatoes and eggplants.

In sharp contrast to this finding, Matsuzoe et al. (1993), report that indeed the two garden egg rootstocks *Solanum torvum* and *Solanum aethiopicum* are poor host of the two most destructive root-knot nematode species (*M. javanica* and *M. incognita*). *Solanum torvum* does offer some tolerance to root knot nematodes but they are however not entirely resistant (Ioannou, 2001).

Generally, research has shown that there are positive correlations or effects of grafting on the agronomic performance (yield) of vegetables hence farmers stand the chance of making more profits from grafted plants as compared to non-grafted plants. According to Pogonyi et al. (2005), in grafted vegetables like tomatoes there is a direct correlation between fruit yield and fruit size a situation which is as a result of the rootstocks being rigorous enough to take up higher amount of water and available nutrients in the soil as compared to non-grafted plants (Lee and Oda, 2003).

Indeed, in grafting for root-knot nematode control in heirloom tomato production Barrett et al. (2012) reported that when susceptible heirloom tomato cultivars (*S. lycopersicum* “Brandywine” and *S. lycopersicum* “Flamme”) were grafted onto two hybrid rootstocks (*S. lycopersicum* “Multifort” and *S. lycopersicum* “Survivor”) with non-grafted and self-grafted as controls, root damage or galling was significantly reduced by 81 percent in the hybrid rootstocks as compared
to the controls. There was however, no clear correlation between root galling and total tomato yields.

### 2.5 Vegetable grafting techniques

A vegetable grafting technique simply refers to any act or process that is able to unite different plant tissues together such that it grows as a composite plant, a successful graft must consist of a scion and a rootstock. The scion refers to a small piece of shoot which have been removed or cut from an existing plant, the scion therefore, has several buds which will sprout after it is attached to a rootstock, in simple terms a scion refers to the upper part of a graft whilst a rootstock refers to the lower part of the graft which eventually develop to form the root system.

Several grafting techniques are used by farmers for various tree crops and vegetable production generally, commercial growers who use eggplants as rootstocks prefer the use of the splice grafting technique as it produces more quality seedlings. Eight grafting methods or techniques are widely used (Clef graft, Offset clef graft, Saddle graft, Side graft, Side veneer graft, Epicotyl graft, Splice graft and Whip and tongue graft). In vegetable grafting splice or tube grafting and cleft grafting are however, used because of the relative ease and flexibility of its use on seedlings with age ranges from three to four weeks (Lee et al., 2010).

With the splice graft technique or method, a slanting cut is made on the scion and the rootstock at a 45°C angle, the cut surfaces are then joined together such that two surfaces come into firm contact ensuring that the cambium layers of the scion and the rootstock are aligned with the help of a grafting tube, the graft could also be held in place with a wax but recently the use of grafting tube has been found to be more efficient and improved to better handle grafts.
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Contrary to the splice graft technique the cleft graft technique involves making a clean horizontal cut on the rootstock 5mm below the cotyledon; a 4 mm vertical incision is then made in the middle of the root stock. The scion is then sharpened into a wedge form and gently inserted into the rootstock were the incision was made.

The selection of a particular grafting method or technique depends on the skill of the person carrying-out the grafting. He or she may choose a technique which they have enough experience in as well the ease with which they can carry it out. Other factors such as the type of crop, the sowing period of the rootstock and the scion is also considered. For instance some farmers prefer using the whip and tongue technique in grafting cucumbers because the seedlings of cucumber are large (hypocotyl length and diameter) which makes easy grafting (Lee, 1994).

The tube grafting method has also been shown to have a high percent graft rate. The grafting of two tomato cultivars (“PG3” and “Beaufort”) using the graft tube method and the clef method resulted in a high percentage graft rate of 79-100% indicating the suitability of the two grafting methods for tomato grafting (Maršić and Osvald, 2004).

2.5.1 Graft union formation

Graft union formation among horticultural crops takes place in several stages, in a particular sequence, only if the two plant parts are compatible. In the first stage the parenchyma cells are
formed between cut surfaces of the scion and rootstock which ensure the interlocking of the
scion and rootstocks, followed by the differentiation of cells and the formation of the cambial
layer of the scion and the rootstock and their eventual connection. Finally the vascular layer is
also formed with the cambial layers laying the foundation for its formation to allow the transport
of nutrients and water (Trinchera et al., 2013). In tomato grafting the formation of the xylem and
phloem vessels occurs after 8 days indicating that graft union formation occurs eight days after
grafting (Fernandez-Garcia et al., 2004).

To achieve a high success (grafting percentage ranging from 79-100%), several factors which
relates to the plants being grafted and its environment must be taken into consideration, these
factors includes, craftsmanship, time of the year, compatibility of the rootstock and scion,
temperature and humidity, age of plant parts, cambial alignment, pressure and avoidance of
desiccation. Poor craftsmanship, graft incompatibility, unfavorable environmental conditions,
diseases and the mismatching of the cambial layer of scion and rootstocks are among the most
reoccurring factors that leads to the failure of a graft. (Hartmann and Kester, 2011).

Graft incompatibility which refers to the inability of a graft union to form between a scion and a
rootstock due to genetic or taxonomic differences is one of the major setbacks in grafting
operations which may have economic implications in terms of grafting percentage and fruit yield
if not checked. The responds of Solanaceous plants to graft incompatibility may differ based on
the combination of the scion and the rootstock that is selected, severe incompatibility has been
observed in tomato/pepper (scion/rootstock) grafts whiles moderate incompatibility have also
been observed in eggplant and tomato (scion/rootstock) grafts in terms of yield and the number
of grafted plants that survived after grafting (Kawaguchi et al., 2008).
The case of severe incompatibility is attributed to the discontinuity of the formation of vascular tissues between the graft, however the case of moderate incompatibility between tomato/eggplant grafts is due to the differences in the requirements for assimilates and mineral nutrients of the scion and rootstock (Kawaguchi et al., 2008).

Figure 1: Effect of botanical relationship on the success of union between stock and scion.

In reference to Figure 1, monocotyledonous plants cannot be grafted because they lack the ability to form cambium layers as compared to dicotyledonous plants, hence they are place outside the
pyramid of grafting success and graft compatibility is therefore achieved among plants which
belong to the same family as well as clones of the same family.

Temperature and relative humidity levels are crucial environmental factors necessary for graft
union formation and acclimatization of grafted plants; hence the regulation of these post grafting
factors will determine the survival rate of the grafts, grafting success and yield. Generally, a
higher relative humidity in the grafting chamber tend to favor grafted tomato plants as it prevents
the tomato grafts from losing too much moisture which may lead to wilting of the plant and
ultimately graft failure (Nobuoka et al., 1996). High humidity in the grafting chamber can be
achieved by misting the chamber regularly with water, the use of plastic polythene to cover the
grafting chamber acts as an insulator which shields the plants from the changes in temperature
and other weather conditions.

An ideal post-grafting operation should therefore include the maintenance of an ideal air
temperature and relative humidity of 25-28°C, 80-90% respectively which will promote a higher
survival rate and the quality of grafted seedlings (Chang et al., 2003). In situations where
temperature levels have exceeded (30-32 °C) the leaf weight, dry weight and fresh weight have
been reported to reduce significantly (Jou et al., 2005).

2.6 Vegetable grafting and fruit quality.

Quality has become the hallmark of consumers who purchase vegetables as part of their daily
dietary requirements; consumers therefore use certain visual and non-visual attributes to
determine the quality of vegetables and fruits in general. Consumers determine the quality of
tomato fruits based on its appearance (size, colour and shape), texture (firmness, mealiness and
juiciness) as well as its flavor and nutritional content (Garg and Cheema, 2011). However,
different market players along the vegetable value chain do have different stands or views on quality, in the fresh tomato market for instance quality is defined based on soluble solids, acidity, sugars, pH and shelf life (Cuartero and Fernandez-Munoz, 1999).

Among farmers and traders of vegetables there is that preference for crop cultivars which exhibit firmness and can withstand mechanical damage whilst in transit to various market centers (Ram, 1999). The term fruit quality which can be defined based on the visual and sensory properties such as colour and sweetness has been found to be controlled by certain inherent genes in some plant cultivar, some of these genes or genetic traits can be bred into new genotypes from other wild species. For instance, in trying to improve on the quality of tomatoes (soluble solids content) genetic traits are introgressed from wild species into new genotypes which then carry this quality traits (Tam et al., 2005).

As resistant rootstocks have the capability to confer their resistance to a scion, rootstock quality traits can also be trans-located to a scion. Grafting has the ability to upgrade the fruit quality of tomatoes (soluble solids content and titrable acidity) and these traits are trans-located from the rootstock to the scion through the xylem of the grafted plant (Flores et al., 2010). In an experiment conducted by Ruiz et al. (2005), where tobacco plants were grafted on tomato rootstocks under optimal conditions the amount of nicotine in the leaves of the scion was eliminated, similarly an increase in carotenoids occurred in tomato cultivars which were grafted onto tomato hybrid when irrigated with saline water (Fernandez et al., 2004).

There are conflicting reports as to whether it is the rootstock or the scion which controls fruit quality attributes. Rouphael et al. (2010) reports that even though scion cultivars or a variety affects the size of fruits, yield as well as other quality characteristics the rootstock can also
modify them. Other report has also questioned if indeed grafting is advantageous or deleterious as far as fruit quality is concern.

In an experiment conducted by Matsuzoe et al. (1996) where tomatoes (Momotaro) were grafted on three Solanum species (S. torvum, S. toxicarum and S. sisymbriifolium) it was observed that there were no significant differences in the quality of tomatoes that were grafted and those that were not grafted in terms of the amount of sugars and organic acid content.

Similarly, Khah et al. (2006), reported that fruits quality attributes such as titrable acidity, pH, total soluble solids and lycopene content were not affected in hybrid tomatoes when grafted. Qaryouti et al. (2007), also reported that not only does grafting have an insignificant effect on the amount of sugars and acid content on grafted plants but also fruit size and shelf life of fruits are not significantly affected by grafting.

The differences in the various research findings concerning whether grafting is advantageous or not could be partly due to the different environments in which the research was conducted (Santoh, 1996). It was observed that differences in methods such as irrigation, fertilization, type of rootstocks and scion combination used and harvest date could contribute to the different accounts been made against or for the argument that grafting is more advantageous or deleterious.

2.7 Screening of Solanum rootstocks against root-knot nematodes

Traditionally field and pot screening have been used to identify plant cultivars that are resistant to root-knot-nematodes as screening of rootstocks against root-knot nematodes is essential for every grafting program as it informs the selection of the right rootstock for grafting. In a field experiment to evaluate the performance of grafted eggplant cultivars on wild Solanum rootstocks
against root-knot nematodes in a field experiment, it was revealed in the data that the wild
*Solanum* rootstocks *Solanum torvum, Solanum sisymbriifolium* and *Solanum khasianum* were
resistant to root-knot nematode when inoculated with 1,000 nematode juveniles (Rahman *et al.*, 2002).

Also, it was observed that the non-grafted plants generally flowered before the grafted plants, a
situation which is attributed to the cut back of the leaves of the scion to reduce transpiration
which slowed down the rate of growth. On the other hand, when Jaiteh *et al.* (2012) screened 33
tomato genotypes for resistance to root-knot nematode under five inoculum levels (100, 500, 1000, 1500 and 2000) the results showed that with increasing inoculum level there was a
 corresponding increase in gall score and fresh root weight.

Equally with an increase in initial inoculum level from 100 to 1500 there was a direct increase in
the population of juvenile nematodes however; at 2000 initial inoculum level there was a
decrease in the population of juveniles. Among the 33 tomato genotypes tested Mongal F1 T-11
had the lowest mean gall score of 3.25 followed by beef master of 3.75 with a reproductive
factor of 0.71 and 0.53 respectively which indicates that the two tomato cultivar were resistant to
root-knot nematode infection. Tomato cultivars that are resistant to root-knot nematode have a
reproductive factor less than one which implies that the plant is able to suppress the reproduction
cycle of the organism once they gain entry into the roots (Karssen and Moens, 2006).

In a pot culture experiment conducted by Dhivya *et al.* (2014), ten (10) *Solanum* plant geneotypes
(*Solanum torvum, S. incanum, S. xanthocarpum, S.aethiopicum, S. sisymbriifolium, S. viarum, S.
vioaceum, Physalis peruviana* TNAU Tomato Hybrid CO-3 and US-618) consisting of eight
wild species and two F1 cultivars were evaluated for their resistance to root knot nematode over
a sixty day period, the results showed that *S. sisymbrifolium* rootstock had the highest shoot fresh weight and dry weight of 103.87 g and 10.44 g respectively. However, in terms of root fresh and dry weight *S. torvum* recorded the highest weight of 54.17 g and 5.53 g respectively compared to the other rootstocks evaluated.

In assessing the *Meloidogyne* populations build up in the various pot cultures per each rootstock, *S. sisymbrifolium, Physalis peruviana* and *S. torvum* recorded the least nematode population building up of 39, 40 and 43 per 200cc of soil with a reproduction factor of 0.71, 0.74 and 0.84 respectively. *Solanum aethiopicum* on the other hand recorded a population build of 88 per 200cc of soil with a corresponding reproductive factor of 1.35.

In conclusion the study found the *Solanum* geneotypes *Solanum sisymbrifolium, Physalis peruviana* and *Solanum torvum* resistant to root-knot nematode (*Meloidogyne incognita*) whilst *Solanum incanum* and *Solanum aethiopicum* were found to be moderately resistant to *Meloidogyne incognita*.

In view of the findings made by (Rahman *et al.*, 2002 and Dhivya *et al.*, 2014), results from field screening of plant cultivars to root-knot nematodes can be misleading sometimes because of the potential variations in nematode populations as well as temperature levels that might not be favourable. In response to this possibility Danso *et al.* (2011) argues that the use of DNA markers in a marker assisted selection programme can be used to detect a specific gene that confers resistance to root-knot nematodes in an array of plant cultivars which is comparatively less strenuous and may be cheaper to run.
2.7.1 Screening of rootstocks for the Mi gene using molecular markers

The resistance offered by plants to the damage caused by root-knot nematodes has been well researched and attributed to the presence of a single dominant gene. The Mi gene confers resistance to a number of root-knot nematode species (\textit{M. incognita}, \textit{M. javanica} and \textit{M. arenaria}) in addition to whiteflies and aphids (Casteel et al., 2006). Several \textit{Solanum} spp. such as \textit{Lycopersicon peruvianum} and \textit{Solanum torvum} have been reported to have this gene which enables the plant to control the feeding activities as well as the reproduction cycle of root-knot nematodes (Messeguer et al., 1991). The mechanism for resistance is based on the fact that in susceptible plants root knot nematodes are able to penetrate the roots and locate the feeding sites within the roots for feeding but in resistance plant varieties or rootstocks feeding sites are not developed hence nematodes are not able to establish a feeding site leading to their death or exit from the roots (Hu et al., 1992).

The Mi gene was first discovered in an accession of a wild \textit{Lycopersicum peruvianum} in South America from which commercial F1 varieties were introgressed with the gene (Cap et al., 1991). This process involves the extraction and detection of the gene using DNA markers and subsequently isolation of the gene for introgression, in isolating the Mi gene several types of research have been conducted to pinpoint the exact location of the gene on the plant chromosome, indeed Willamson et al. (1994) reports that multiple markers have been extensively used which indicates that the Mi gene can be located on the short arm of chromosome 6.

In other related research conducted using the positional cloning approach to isolate gene with linked traits and the subsequent sequencing of the DNA, Kaloshian et al. (1998) reported that the sequencing analysis showed two genes which were identical to each (Mi-1.1 and Mi-1.2) which
also confers resistance to three species of root-knot nematodes namely *M. arenaria*, *M. javanica* and *M. incognita*.

Several DNA markers have been developed for the detection of the Mi gene in plants using polymerase chain reactions (PCR) amplifications, for instance in an experiment carried out by Devran *et al.* (2004) where Mi gene specific primers C1/2 (5’-cagtgagtggaaggtgtaa-3’) and C2S4 (5’-ctaacaggaatctcatacagggg-3’) were used to amplify the Mi gene following the CETAB protocol to screen F2 tomato Plants for the root-knot nematode resistance gene. A 1.6 kb amplification product was amplified in the resistance F2 plants containing the Mi-1.2 gene in the 3′ region however, it was found to be absent in the susceptible F2 plants tested.

Similarly, in another study conducted by Goggin *et al.* (2006) where the Mi-1.2 gene was introgressed into *Solanum melongena* to assess the performance of the gene in conferring resistance to root-knot nematode *Meloidogyne javanica* and aphids, the results of the study showed that the transgene eggplant was able to offer resistance to *Meloidogyne javanica* and not aphids. In confirming the present of the Mi-1.2 gene in the transgene eggplant a reverse-transcription polymerase chain reaction assay with the Mi specific primers C2D1 (5′-ctgggttcagttcttaacaagggg-3’) and C2S4 (5’-ctaacaggaatctcatacagggg-3’) amplified a single PCR band of 915bp in the transgene *Solanum melongena* confirming the presence of the gene compared to a non-transgene *Solanum melongena* which did not produce any PCR product.

A study carried out in Morocco by Mehrach *et al.* (2007) to detect the Mi-1.2 gene which confers resistance to root-knot nematodes in 14 begomovirus-resistant breeding lines with known resistant and susceptible cultivars in a two steps PCR method. They reported that in using the primers PM3Fb/PM3Rb, REX primers and PMiF3/PMiR3, the primer pairs PM3Fb/PM3Rb and REX primers in a multiplex PCR amplified a band of 720-bp for both susceptible and resistance
varieties however, the resistant varieties tested (Motelle and Better Boy) produce an additional band of 500-bp indicating the presence of the Mi gene in those cultivars.

In distinguishing between heterozygous and homozygous plant cultivars with the Mi-1.2 gene the primer pairs of PMiF3/PMiR3 amplified a single unique band of 350-bp for the susceptible cultivars (Moneymaker and Daniella). On the other hand, a 550-bp and 350-bp fragment for both the homozygous and heterozygous plant resistant cultivars “Motelle” and “Better Boy” was amplified respectively.

Resistance to rootknot nematode damage by plants is not absolute as other factors such as temperature may affect the functioning of the gene that confers the resistance in the plant. Several authors such as Dropkin, (1969), have reported that above soil temperatures of 28°C the Mi gene which controls root knot nematodes deteriorates irreversibly similarly Mi virulent rootknot nematode populations are able to break down and overcome the resistance conferred by the Mi gene rendering plants with the gene susceptible.

2.8 Rootstock regrowth (Suckering)

Rootstock regrowth or adventitious bud growth is a common observation in vegetable grafting it refers to the apical meristem tissue development on the rootstock due to an incomplete removal of the cotyledon of the rootstock.). Rootstock regrowth is a major concern because of its potential in increasing the labor cost of producers who have to employ work hands to pinch and removed the adventitious buds (Guan, 2012). Rootstock regrowth can also lead to scion abortion which ultimately leads to graft failure; a vigorous rootstock regrowth competes with the scion for water and nutrients as well as enveloping the scion, a situation which can lead to a drastic decrease in yield (Bausher, 2011).
2.9 Cost implications of using grafted tomato transplants

Increasingly consumers and environmentalist are raising concerns over the use of fumigants and other chemical based treatments of pest and diseases especially in the cultivation of vegetables as residues of this chemical can cause serious health implications. Grafting of plants in Solanacae and cucurbits is a sure way of overcoming these concerns however, growers are equally concern about the cost component associated with grafted plants, a factor which prevents some farmers from adopting this know how (Barrett et al., 2012).

According to Djidonou et al. (2013), a positive or negative net return is mainly dependent on the cost of producing the grafted plants and the prevailing market price for the tomato fruits that will be produced. Falling tomato prices coupled with high input cost for raw materials needed for grafting may result in some negative net returns. He also goes on to argue that the net returns are also sensitive to the vigorousness of the rootstock and that the higher the marketable fruits the higher the net returns.

Generally labor cost represents a small proportion of the total cost of grafting, Rivard et al., 2012 states that the bulk of the cost goes to the purchase of root stock seeds which are specially breed and forms 36% of the total cost, he goes on to state that apart from the cost of seeds other inputs such as grafting clips and building a humidity adds up to the additional cost.

In view of the cost components grafted transplants are more expensive to produce per plant than non-grafted plants. Therefore, lowering the cost of rootstock can easily boost the rate at which farmers adopt this technology (Barrett et al., 2012).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area.

Three experiments consisting of a greenhouse pot experiment, a field pot experiment and a field experiment were conducted at the Sinna garden of the Department of Crop Science, University of Ghana and a farmer’s field at Haatso a suburb in Accra-Ghana.

3.2 Experiment 1: Detection of Mi gene which confers resistance to root knot nematodes in Solanum rootstocks (PCR experiment)

3.2.1 Sources of seeds and preparation

Seeds of Solanum lycopersicum “Mongal F1”, Solanum lycopericum “Samrudhi F1” Solanum macrocarpon and Solanum aethiopicum were sourced from Agriseed, East-West Seeds and the Department of Crop Science, University of Ghana respectively. The seeds were nursed in 98-cell seed trays containing planting media prepared from 2:2:1 v/v rice husk, sawdust and manure. Two weeks after the emergence of seedlings, N-P-K: 15:15:15 (6 g/L) nutrient solution was applied by immersing the base of the seed tray into a bath containing the nutrient solution for 5 minutes. A total of 288 four-week old seedlings were then transplanted singly into plastic pots measuring 25 cm high, 20 cm diameter at the top and 20 cm diameter at the base with perforated holes at the base and filled with 6 kg of sterilized and sieved (2 mm) top soil.

3.2.2 Fertilizer application

A starter solution formulated by dissolving 6 g of N-P-K: 15:15:15 in 1L of water was applied at a rate of 50 ml per plant after transplanting. A compound fertilizer N.P.K (15-15-15) was also
applied in split applications at a rate of 6 g per plant two weeks after transplanting followed by 3.0 g of sulphate of ammonia four weeks after the transplanting date.

3.2.3 **Inoculation of plants with nematode juveniles**

Root-knot nematode inoculums (Juveniles) were prepared by extracting nematode eggs from the roots of infested plants and hatched into juveniles (Hussey and Barker, 1973) which were then concentrated into the various inoculum levels (500, 1,000 and 5,000). The plants (rootstocks) were then inoculated with the juveniles by making a small hole or depression in the soil at the base plants after which the required inoculum level was gently poured into the hole and covered.

3.2.4 **Experimental design**

The layout for this experiment was a 4 x 4 factorial pot experiment with a control arranged in a split plot design with three replicates.

**Treatment structure**

*Solanum aethiopicum*

T1: Control – *Solanum aethiopicum* + no root knot nematode inoculum.

T2: *Solanum aethiopicum* + 500 root knot nematode juveniles.

T3: *Solanum aethiopicum* + 1000 root knot nematode juveniles.

T4: *Solanum aethiopicum* + 5000 root knot nematode juveniles.

*Solanum macrocarpon*

T1: Control – *Solanum macrocarpon* + no root knot nematode inoculum.
T2: *Solanum macrocarpon* + 500 root knot nematode juveniles.

T3: *Solanum macrocarpon* + 1000 root knot nematode juveniles.

T4: *Solanum macrocarpon* + 5000 root knot nematode juveniles.

*Solanum lycopersicum “Mongal F1”*

T1: Control – *Solanum lycopersicum “Mongal F1”*+ no root knot nematode inoculum.

T2: *Solanum lycopersicum “Mongal F1”*+ 500 root knot nematode juveniles.

T3: *Solanum lycopersicum “Mongal F1”*+ 1000 root knot nematode juveniles.

T4: *Solanum lycopersicum “Mongal F1”*+ 5000 root knot nematode juveniles.

*Solanum lycopersicum “Samrudhi F1”*

T1: Control – *Solanum lycopersicum “Samrudhi F1”*+ no root knot nematode inoculum.

T2: *Solanum lycopersicum “Samrudhi F1”*+ 500 root knot nematode juveniles.

T3: *Solanum lycopersicum “Samrudhi F1”*+ 1000 root knot nematode juveniles.

T4: *Solanum lycopersicum “Samrudhi F1”*+ 5000 root knot nematode juvenile.

3.2.6 **Root-knot nematode extraction and counting**

Root-knot nematodes were extracted from soil samples within each pot inoculated with nematodes using the sieving and sucrose centrifugation method as described by (Jenkin, 1964).

1. 200 cc of soil was taken from each pot around the roots of the rootstock into 1L beaker and topped up with distilled water to the 800 cm³ mark and crushed thoroughly for 2
minutes. The suspension was then allowed to settle for 5-10 minutes after which the upper the ¾ of the suspension was poured through 1 mm, 71µm and 36 µm mesh sieves nested on top of each other respectively.

2. The nematodes were then collected on the 36 µm mesh and washed with a fine spray of water into a centrifuge tube.

3. The tubes were then placed in a centrifuge and span at 1700 rad/s for 5 minutes.

4. The supernatant was gently discarded to about 1 cm above the pellets formed at the bottom of the falcon tube followed by the addition of 454 g/L sucrose solution to the pellet formed (twice the volume of the pellet formed).

5. The sucrose solution together with the pellet was centrifuged again at 1000 rad/s for 1 minute and the upper ¾ of the solution poured through 71µm and 36µm mesh nested together.

6. A sufficient amount of distilled water was used to wash off the sucrose from the nematodes collected on the 36 µm mesh and then poured into a labeled falcon tube with the aid of fine spray of water.

7. The extracted nematode was then observed in a viewing dish using a compound microscope at a magnification of 40x. Counting was done with the aid of a tally-counter and recorded.

3.2.6.1 Counting of root-knot nematode eggs

Extraction and counting of root-knot nematode eggs were carried out after 6 weeks of inoculating the plants (selected rootstocks) and 12 weeks after harvesting to determine the rate of root-knot multiplication within the rootstocks as well as the reproductive factor.
Three pot plants were randomly selected from each treatment level for root-knot nematode egg count after six weeks and 12 weeks, the roots of the plants were cut weighed and eggs extracted from 1g of root mass using the modified method as described by Hussey and Barker, (1973). The eggs extracted were counted under a compound microscope at a magnification of 100x and recorded.

3.2.7 Scoring of root-knot nematode galls

Roots of the rootstocks were washed and allowed to air dry for 5 minutes. The galls on each root were scored using the score chart by Bridge and Page (1980). Rating of the gall was done according to the rating scale of 0-10 were 0 - no knots on the roots to 10 - all roots severely knotted.

3.2.8 Establishing the reaction of Solanum spp. rootstocks against root-knot nematode

The mean gall scores, egg count per gram of root, and reproductive factors were used to establish the resistance level of the rootstocks. Rootstocks were considered as resistant when the root gall index is ≤ 2 and susceptible when the root gal index > 2 and also RF<1 and RF>1 for resistant and susceptible respectively.

3.3.2 Data collected

1. Plant height (cm), Plant girth (mm) and plant chlorophyll content

2. Initial and final nematode population in 200cc of soil/pot

3. Root galling score for six and twelve weeks after applying treatments

4. Fresh shoot and root weight of recorded plants (g)

5. Dry shoot and root weight of recorded plants (g)
6. Root-knot nematode eggs from 1g of roots

3.3.3 Statistical analysis

Analysis of variance (ANOVA) for the data collected was carried out using Genstat statistical package (version11). Where there were significant differences the Least Significant Difference test (LSD) was used to determine differences among the means for the various parameters studied. Significance was defined at $P<0.05$ and where necessary data was transformed for normality.

3.3 DNA extraction

DNA was extracted from young leaf tissues of the four *Solanum* genotypes (*Solanum lycopersicum* “Mongal F1”, *Solanum lycopersicum* “Samrudhi F1” *Solanum macrocarpon* and *Solanum aethiopicum*) using the E.Z.N.A.® SP Plant DNA Kit (Omega bio-tek) following manufacturer’s instructions.

3.3.1 Genomic DNA amplification

The codominant gene specific markers PMiF3-R3 (Mehrach *et al.*, 2007) and C2D1/C2S4 (Goggin *et al.*, 2006) were used to detect the resistance gene Mi1-2. PCR was carried out in a 25 μL reaction volume containing PCR Buffer, 5 pmol of each primer, 0.2 mM dNTP, 2 mM MgCl2, 20 ng of template DNA and 1 Unit Taq DNA Polymerase. The PCR thermocycler was programmed for 10 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C (all primers) and 1 min at 72°C and ended with 5 min incubation at 72°C. Five μL of each reaction was separated by electrophoresis in TaE buffer1.5% agarose gel to ascertain whether or not the PCR amplification was successful.
3.4 Experiment 2: Effect of grafting *Solanum lycopersicum* “Pectomech” on *Solanum macrocarpon*, *Solanum lycopersicum* “Mongal F1” and *Solanum aethiopicum* on fruit quality, shelf life, yield and agronomic parameters in nematode infested soils (Field Pot experiment).

3.4.1 Planting materials used

Seeds of *Solanum lycopersicum* "Pectomech", *Solanum macrocarpon*, *Solanum lycopersicum* “Mongal F1”, *Solanum lycopersicum* “Samrudhi F1” and *Solanum aethiopicum* were used in this experiments. The *Solanum lycopersicum* “Pectomech” was used as scion and *Solanum macrocarpon*, *Solanum lycopersicum* “Mongal F1”, *Solanum lycopersicum* “Samrudhi F1” and *Solanum aethiopicum* used as rootstocks.

3.4.2 Raising of seedlings for grafting

Seedlings for this experiment were raised in 98-cell seed trays containing planting media prepared from 2:2:1 v/v rice husk, sawdust and manure. The growth medium was sterilized in an oven at a temperature of 70 degrees over a three-day period. The seeds of, *Solanum macrocarpon* and *Solanum aethiopicum* were sown one week before that of the *Solanum lycopersicum* “Mongal F1”, *Solanum lycopersicum* “Samrudhi F1” and *Solanum lycopersicum* “Pectomech” this was done to obtain the same plant girth for easy grafting due to the differences in their growth rate. The *Solanum lycopersicum* “Pectomech” which was used as a scion was nursed for three weeks together with *Solanum lycopersicum* “Mongal F1”, *Solanum lycopersicum* “Samrudhi F1” whilst *Solanum macrocarpon* and *Solanum aethiopicum* nursed for four weeks.
Figure 2: Seedlings of *Solanum lycopersicum* “Pectomech” (A) and *Solanum aethiopicum* (B) at one-week interval.

### 3.4.3 Fertilizer application

A starter solution formulated by dissolving 6 g of N-P-K: 20:20:20 in 1L of water was applied at a rate of 50 ml per plant once every two weeks after germination till the seedlings were ready for grafting.

### 3.4.4 Grafting of seedlings

The grafting of seedlings was carried out by hand, using the cleft method when the scions developed to a stem girth of 5 mm and the rootstocks develop to a stem girth of 7 mm. The rootstocks were watered and fertilized with N.P.K 20:20:20 24 hours before grafting to prevent water and nutrient stress during the healing process.
3.4.5 Method of grafting

Brand new razor blades and grafting clips were sterilized in a 10% bleach solution before using them in the grafting process to prevent pathogenic contamination. The graft was made by severing the stem of the rootstock seedlings 5mm below its cotyledon, a vertical slit was then made in the middle of the rootstock (4 mm deep). The tomato scion *Solanum lycopersicum* “Pectomech” was also severed below the cotyledon and the leaves defoliated leaving only the apical leaves to reduce the excessive loss of moisture by the scion, the cut surface was then shaped into a wedge form and gently inserted into the vertical slit on the rootstock. The graft was then firmly held in place with a grafting clip and quickly placed in a humidity chamber.

3.4.6 Healing of graft unions in a humidity chamber

A healing or humidity chamber was constructed using a Styrofoam fish box measuring (Length = 60 cm, Breath = 40 cm and High = 20 cm). The top of the box was covered with a white transparent plastic using thumb tracks to create a humid environment inside the box.

Figure 3: A grafted tomato plant showing the cleft graft method
Before the grafted plants were placed in the Styrofoam box the inner base of the box was sprayed with water using a squirt bottle, the grafted plants were then placed in the box and quickly covered with the transparent plastic.

The whole set up was then covered with black plastic for three days to cut down on the amount of light entering the box and also to reduce transpiration. The humidity chamber was opened daily for 60 seconds in the morning and evening to allow the hot air build up in the chamber to escape; this routine was repeated for four consecutive days till the period of acclimatization.

3.4.7 Acclimatization of grafted plants

After the grafted plants had healed (over an eight day period) small holes were made in the white plastic covering to gradually reduce the humidity in the healing chamber to the prevailing environmental conditions over a week period and subsequently the entire plastic covering was also removed. The plants were then fertilized and kept in the green house for another week before transplanting into pots.
Figure 4: A Styrofoam humidity chamber set-up: A - Side elevation of humidity chamber; B - Top elevation of humidity chamber; C - Grafted plants undergoing acclimatization; D - Seedlings of grafted pectomech tomato on S. aethiopicum; E - Seedlings of grafted pectomech tomato on S. macrocarpon.
3.4.8 Soil preparation, plastic pot filling and transplanting of grafted plants

Top soil from the University of Ghana Sinna Garden was collected and sieved through a 2 mm sieve. The soil is described as Haatso series which is also described as entisol and sandy clay loam in texture. Bulk samples of the soil were sterilized at 80°C for two days for all weeds, nematodes, and other soil pathogens to be killed in an electric oven. The soil samples were then left overnight to cool and then filled into plastic pots (25 cm high, 20 cm diameter at the top and 20 cm diameter at the base with perforated holes at the base.

The weight of the soil media in the plastic pots was 6 kg and it was watered and allowed to drain overnight to attain field capacity prior to transplanting. The seedlings were transplanted into the plastic pots to a depth of 5 cm, there were five plants per treatment and a total of 120 seedlings transplanted. Seedlings were watered after transplanting.

3.4.9 Fertilizer application

A starter solution formulated by dissolving 6g of N-P-K: 20:20:20 in 1L of water was applied at a rate of 50 ml per plant after transplanting. A compound fertilizer N.P.K (15-15-15) was also applied in split applications at a rate of 6 g per plant two weeks after transplanting followed by 3.0 g of sulphate of ammonia four weeks after the transplanting date.

3.5 Root-knot nematode inoculum and inoculation of plants with nematode juveniles

Root-knot inoculum (Juveniles) was prepared by extracting nematode eggs from the roots of infested okra plants and hatched into juveniles (Hussey and Barker, 1973), the hatched root-knot nematode juvenile population were estimated and then concentrated into the various inoculum levels (500, 1,000 and 5,000). The plants (rootstocks) were then inoculated with the juveniles by
making a small hole or depression in the soil at the base of the plant after which the required inoculum level was gently poured into the hole and covered in the pots.

3.5.1 Experimental design

The experimental layout used for this experiment was a 2 x 4 factorial pot experiment with a control arranged in a split plot design with three replicates.

3.5.2 Treatment structure

*Solanum lycopersicum* “Pectomech” grafted on two *Solanum* rootstocks.

- *Solanum aethiopicum* (SA)
- *Solanum macrocarpon* (SM)

Three levels of root-knot nematode inoculum (Juvenile) plus a control.

- Control (No juveniles in ml of water)
- 500 juveniles per ml of water.
- 1,000 juveniles per ml of water.
- 5,000 juveniles per ml of water.

T1: Control – Pectomech // *Solanum aethiopicum* + no root knot nematode inoculum.

T2: Pectomech // *Solanum aethiopicum* + 500 root knot nematode juveniles.

T3: Pectomech // *Solanum aethiopicum* + 1000 root knot nematode juveniles.

T4: Pectomech // *Solanum aethiopicum* + 5000 root knot nematode juveniles.
Solanum macrocarpon

T1: Pectomech // Control – Solanum macrocarpon + no root knot nematode inoculum.

T2: Pectomech // Solanum macrocarpon + 500 root knot nematode juveniles.

T3: Pectomech // Solanum macrocarpon + 1000 root knot nematode juveniles.

T4: Pectomech // Solanum macrocarpon + 5000 root knot nematode juveniles.

3.5.3 Assessment and Scoring of root-knot nematode galls

Roots of the rootstocks were washed and allowed to air dry for 5 minutes. The galls on each root were scored using the score chart by Bridge and Page (1980). Rating of the gall was done according to the rating scale of 0-10.

0 - No knots on the roots.
1 - Few small knots, difficult to find.
2 - Small knots only but clearly visible. Main roots clean.
3 - Small large knots visible. Main roots clean.
4 - Large knots predominate but main roots clean.
5 - 50% of roots affected. Knotting on some roots. Reduced root system.
6 - Knotting on main roots.
7 - Majority of roots knotted.
8 - All main roots, including tap root, knotted. Few clean roots visible.
9 - All roots severely knotted. The plant usually dying.
10 - All roots severely knotted. No root system. The plant usually dead.
3.5.4 Data collected

1. Plant height (cm), Plant girth (mm), leaf Chlorophyll content
2. Root galling score, twelve weeks after applying root-knot nematode treatments
3. Fresh shoot and root weight of recorded plants (g)
4. Dry shoot and root weight of recorded plants (g)
5. Root-knot nematode eggs from 1g of roots
6. The Total Titrable Acids (TTA)
7. Total Soluble Solids (TSS)
8. Fruit firmness
9. The number of locules
10. Fruit pericarp thickness
11. pH
12. Fruit shelf life
13. Days to 50% flowering

3.5.4.1 Method for the determination of Total Soluble Solids of tomato fruits

The TSS of the tomato fruits was determined using a digital hand refractometer (Hanna© refractometer 96801) at room temperature. A drop of distilled water was first placed on the illumination plate and zeroed after which the water was wiped off the illumination plate. A drop of tomato juice was squeezed out of the tomato samples and placed on the illumination plate; the percent brix was then read from the LCD monitor on a scale of 0 to 85% brix. The process of zeroing was repeated preceding each sample reading.
3.5.4.2 Method for the determination of pH

The pH of the tomato fruits was determined using a 3330 pH meter which was buffered at a pH of 7. Samples of the tomato fruits were blended and sieved to obtain the juice and the pH determined by dipping the pH meter into the juice.

3.5.4.3 Method for the determination total titrable Acidity

10ml of tomato juice samples was squeezed and transferred into a 125ml conical flask, 25ml of de-ionized water was then added to the 10 ml of tomato juice in the conical flask. Three drops of phenolphthalein indicator were then added and titrated with 0.1 N of NaOH till a colour change was observed and the titre value recorded.

3.5.4.3 Method for the determination of the number of locules

Samples of the tomato fruits were transversely cut opened and the number of locules in one half of the fruit counted.

3.5.4.4 Method for the determining fruit firmness

The firmness of the tomato fruit samples was determined using a hand penetrometer with an 11mm plunger, the plunger was pressed against the flesh of the fruit until it reached marked fixed depth on the piston. The display value which represents the resistance offered by the pericarp of the fruit to the plunger was recorded, two readings were taken from each fruit and the average recorded in Newton.

3.5.4.5 Method for measuring plant height (cm)

Plant height was taken two weeks after transplanting and repeated weekly until the last harvest. The heights of the plants were taken from the soil level to the highest tip of the grafted plants and a meter rule used to record the height.
3.5.4.6 Method for measuring stem girth (cm)

The girths of the grafted plants were taken at four different points along the stem at 5 cm above the soil with an electronic caliper and the average taken.

3.5.4.7 Method for measuring chlorophyll content of leaves.

The chlorophyll content of the grafted plants was taken with a SPAD meter, the forceps sensor was clamped to the leaf and the chlorophyll content taken.

3.5.4.8 Method for determining plant fresh shoot weight (g)

The upper portion of the grafted plants above the soil level was chopped up after harvest and weighed with an electronic balance.

3.5.4.9 Method for determining plant dry shoot weight (g)

This was determined by chopping up the upper portion of the plant above the soil level and drying it in an electronic oven at 80 degrees Celsius for 72 hours to constant weight.

3.5.4.10 Number of days to flowering

The records plants were observed and the number of days to flowering determined.

3.5.5 Yield per plant (g)

The weight of fruits per plant was taken as cumulative weight, the total sum of matured fruits per record plant was weighed and the total fruit weight divided by the number of plants and the mean computed.

3.5.5.1 Fresh root weight (g)

The roots of the record plants were gently up rooted and the fresh weight weighed.
3.5.5.2 Dry root weight (g)

The root of the record plants was dried in an electronic oven to at 80 degrees Celsius for 72 hours and then weighed to constant weight.

3.5.5.3 Cultural practices

3.5.5.3.1 Weed Control
Weeds were controlled by hand picking weeds growing in the pots whilst hoeing every two weeks around the treatment pot till the completion of the experiment.

3.5.5.3.2 Staking
Grafted plants were staked with wooden sticks one month after transplanting to support the stem of the plant and fruits.

3.5.5.3.3 Pest Control
Cydim Super, an emulsifiable concentrate containing 36 g cypermethrin and 400 g dimethoate per liter, was applied at 2m/liter every two weeks with CP 15 Liter Knapsack sprayer to control insect pest depending on the level of infestation. Pest observed on the field included caterpillars and white flies (*Bemisia tabaci*).

3.5.5.3.4 Irrigation
Watering was done mainly using a 15 L watering can. Watering was done twice a day, early in the morning and in the evenings depending on the prevailing weather conditions.

3.5.5.3.5 Stirring
The soil was stirred with a hand trowel to aerate the growth media every two weeks.

3.5.5.4 Statistical analysis

Analysis of variance (ANOVA) for the data collected was carried out using Genstat statistical package (v.11). Where there were significant differences the Least Significant Difference test
(LSD) was used to determine differences among the means for the various parameters studied. Significance was defined at P<0.05 and where necessary data was transformed for normality.

3.6 EXPERIMENT 3: Effect of grafting Pectomech on Solanum macrocarpon and Solanum aethiopicum on fruit quality, shelf life, yield and agronomic parameters (Field experiment)

3.6.1 Planting materials used and raising of grafted seedlings

Seeds of Solanum lycopersicum “Pectomech”, Solanum macrocarpon, and Solanum aethiopicum were used in this experiment. The Solanum lycopersicum “Pectomech” was used as scion and Solanum macrocarpon, and Solanum aethiopicum used as rootstocks. The Solanum lycopersicum “Pectomech” seedlings which were used as scion was nursed for three weeks whilst Solanum macrocarpon and Solanum aethiopicum were nursed for four weeks.

Grafting of seedlings was carried out by hand, using the cleft method at a stem girth of 5 mm and 7 mm for both scion and rootstock respectively. The rootstocks were hardened off and fertilized with N.P.K 20:20:20 over a two-week period before being transplanted to the field.

3.6.2 Land preparation

The experimental field was hoed to remove all weeds and also leveled to give it a good tilt. The land was then lined and pegged into three main plots measuring 12 m x 1.6 m with a pathway of 0.3 m. Each main plot was sub-divided into three mini plots measuring 4 m x 1.6 m.

3.6.3 Transplanting of grafted plants

The grafted plants were planted at a spacing of 80 x 40 cm. A total of 270 plants consisting of 90 non-grafted Pectomech, 180 pectomech grafted on Solanum macrocarpon and Solanum aethiopicum rootstock respectively. A starter solution of (5 g/L) N.P.K (15-15-15) was applied at
a rate of 100 ml per plant to the base of the plant whilst making sure that the nutrient solution did not touch the leaves.

### 3.6.4 Fertilizer application

A compound fertilizer N.P.K (15-15-15) was applied in split applications at a rate of 6 g per plant two weeks after transplanting followed by side dressing with 3.0 g of sulphate of ammonia at the rate 312 kg per hectare four weeks after transplanting.

### 3.6.5 Weed Control

Weeds were controlled by hoeing every two weeks till the completion of the experiment.

### 3.6.7 Staking

Grafted plants were staked with wooden sticks one month after transplanting to support the stem of the plant and fruits.

### 3.6.8 Pest Control

Cydim Super, 36 g cypermethrin and 400 g dimethoate per liter, was applied at 2 m/liter every two weeks with CP 15 (Liter) Knapsack sprayer to control insect pest depending on the level of infestation. Pest observed on the field included caterpillars and white flies (*Bemisia tabaci*).

### 3.6.9 Irrigation

Watering was done mainly using a 15 L watering can. Watering was done twice a day, early in the morning and in the evenings depending on the prevailing weather conditions.

### 3.6.10 Treatment structure

T1: Control – Pectomech non-grafted.

T2: Pectomech grafted onto *Solanum macrocarpon*. 
T3: Pectomech grafted onto *Solanum aethiopicum*.

3.7 Experimental design

The experiment design used for this experiment was a randomized complete block design with three replicates.

3.7.1 Statistical analysis

Analysis of variance (ANOVA) for the data collected was carried out using Genstat statistical package (v.11). Where there were significant differences the Least Significant Difference test (LSD) was used to determine differences among the means for the various parameters studied where necessary data was transformed for normality.
CHAPTER FOUR

4.0 RESULTS

4.1 Mean gall score, nematode count /200cc of soil, egg count per gram and the reproductive factor of Solanum spp. Rootstocks in nematode infested soils (Greenhouse pot experiment).

Table 1: Interactive effect between Solanum spp. rootstocks and inoculum densities of Meloidogyne spp. on final nematode count, mean gall scores, egg count per gram of root and reproductive factors 6 weeks after inoculation.

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>X</th>
<th>Inoculum density</th>
<th>Mean gall score (1-10)</th>
<th>Nematode count/200cc of soil (Pf)</th>
<th>Egg count per gram of roots</th>
<th>Reproductive factor (Pi/Pf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon</td>
<td>500</td>
<td>0.51</td>
<td>48.30</td>
<td>6.50</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>SA</td>
<td>500</td>
<td>0.22</td>
<td>125.6</td>
<td>9.60</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>SAM</td>
<td>500</td>
<td>0.02</td>
<td>46.60</td>
<td>2.60</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>SM</td>
<td>500</td>
<td>0.38</td>
<td>107.9</td>
<td>9.60</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Mon</td>
<td>1000</td>
<td>1.11</td>
<td>62.20</td>
<td>12.8</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>SA</td>
<td>1000</td>
<td>0.67</td>
<td>192.3</td>
<td>12.2</td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>SAM</td>
<td>1000</td>
<td>0.50</td>
<td>124.3</td>
<td>7.00</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>SM</td>
<td>1000</td>
<td>0.44</td>
<td>86.10</td>
<td>15.3</td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>Mon</td>
<td>5000</td>
<td>0.33</td>
<td>91.60</td>
<td>7.20</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>SA</td>
<td>5000</td>
<td>0.72</td>
<td>158.4</td>
<td>11.9</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>SAM</td>
<td>5000</td>
<td>1.00</td>
<td>84.70</td>
<td>8.50</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>SM</td>
<td>5000</td>
<td>0.55</td>
<td>206.0</td>
<td>9.60</td>
<td></td>
<td>0.02</td>
</tr>
</tbody>
</table>

**LSD (P < 0.05)** | **NS** | **NS** | **NS** | **0.09**

SAM = Solanum lycopersicum “Samrudhi F1”, Mon = Solanum lycopersicum “Mongal F1”, SM = Solanum macrocarpon, SA = Solanum aethiopicum, Pi = Initial nematode population, PF = Final nematode population.

There were no significant interaction effects between the Solanum spp. rootstocks and the inoculum density of the Meloidogyne spp. on the mean gall scores, egg count per gram of roots and nematode count per 200 cc of soil six weeks after inoculation (Table1).
The interaction effect between the rootstocks *Solanum macrocarpon* and *Solanum lycopersicum* “Mongal F1” and the inoculum density 5000 recorded the least reproductive factor of 0.02, the combination effect between *Solanum aethiopicum* and the inoculum density 500 on the mean reproductive factor (0.25) was significantly higher than the combined effect between *Solanum lycopersicum* “Mongal F1” and *Solanum lycopersicum* “Samrudhi F1”. *Solanum aethiopicum* and the inoculum level 500 recorded the highest (P mean reproductive factor (0.25) (Table 1).

There were significant differences in gall scores and reproductive factor but not in nematode count per 200 cc of soil. There was an increasing inoculum effect on gall scores from 500 to 1000. The inoculum level 1000 recorded the highest gall scores (0.68) which was significantly higher than the gall score of the inoculum level 500. There was a direct significant decrease in reproductive factors from 500 to 5000. The inoculum density 500 recorded the highest reproductive factor (0.16) whilst the inoculum density 5000 produced the lowest reproductive factor (0.03) (Table 2).

**Table 2: Influence of initial inoculum densities of Meloidogyne spp. on mean gall score, nematode count per 200 cc of soil and reproductive factor 6 weeks after inoculation.**

<table>
<thead>
<tr>
<th>Initial inoculum levels</th>
<th>Gall score</th>
<th>Nematode count per 200 cc of soil</th>
<th>Reproductive factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>0.28</td>
<td>82.1</td>
<td>0.16</td>
</tr>
<tr>
<td>1000</td>
<td>0.68</td>
<td>116.2</td>
<td>0.12</td>
</tr>
<tr>
<td>5000</td>
<td>0.65</td>
<td>135.2</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>LSD (P &lt; 0.05)</strong></td>
<td><strong>0.17</strong></td>
<td><strong>NS</strong></td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>
There were no interaction effects between Solanum spp. rootstock and the inoculum densities of Meloidogyne spp. on the fresh root weight, dry root weight, fresh shoot weight and dry root weight six weeks after inoculation.

The inoculum density 500 recorded the highest shoot fresh weight (51.8) which was significantly higher compared to the inoculum density 1000 which recorded the lowest shoot fresh weight (36.9). There was also significant decrease in shoot fresh weight from 500 to 1000 (Table 3).

Table 3: Influence of initial inoculum densities of Meloidogyne spp. on mean shoot fresh weight, root fresh weight, root dry weight and shoot fresh weight 6 weeks after inoculation.

<table>
<thead>
<tr>
<th>Initial inoculum levels</th>
<th>Shoot fresh weight/(g)</th>
<th>Root fresh weight/(g)</th>
<th>Shoot dry weight/(g)</th>
<th>Root dry weight/(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>51.8</td>
<td>1.98</td>
<td>6.51</td>
<td>1.98</td>
</tr>
<tr>
<td>1000</td>
<td>36.9</td>
<td>2.68</td>
<td>8.63</td>
<td>2.68</td>
</tr>
<tr>
<td>5000</td>
<td>50.0</td>
<td>5.32</td>
<td>7.15</td>
<td>5.32</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>10.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

There were significant interactive effects between the rootstock Solanum lycopersicum “Mongal F1”, and the inoculum density 5000 on the mean gall score (0.7) which significantly differed to the rootstocks Solanum lycopersicum “Samrudhi F1” (0.1), Solanum aethiopicum, (0.2) and Solanum macrocarpon (0.0). The interactive effect between the rootstock Solanum macrocarpon and the inoculum density 500, 1000 and 5000 recorded the least reproductive factors of 0.01, 0.00 and 0.00 respectively. The interactive effect between the rootstocks and the various levels of inoculum yielded reproductive factors which were less than one. The interactive effect between the rootstocks Solanum lycopersicum “Samrudhi F1”, Solanum lycopersicum “Mongal
F1” and the inoculum density 500, 1000 and 5000 yielded the least nematode count per 200 cc of soil 1.63, 1.09 and 0.89 respectively (Table 4).

Table 4: Influence of Solanum spp. and inoculum densities of Meloidogyne spp. on final nematode count, mean gall scores, egg count per gram of roots, reaction of rootstocks and reproductive factors of root knot nematodes 12 weeks after inoculation.

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>Initial Inoculum densities (Pi)</th>
<th>Mean gall score (1-10)</th>
<th>Nematode count per 200 cc of soil (Transformed)*</th>
<th>Egg count per gram of roots (Transformed)*</th>
<th>RF</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon</td>
<td>500</td>
<td>0.0</td>
<td>41.28(5.94)</td>
<td>5.94(2.4)</td>
<td>0.08</td>
<td>R</td>
</tr>
<tr>
<td>SA</td>
<td>500</td>
<td>0.2</td>
<td>60.72(7.78)</td>
<td>5.11(2.25)</td>
<td>0.12</td>
<td>R</td>
</tr>
<tr>
<td>SAM</td>
<td>500</td>
<td>0.1</td>
<td>4.00(1.63)</td>
<td>5.44(2.32)</td>
<td>0.22</td>
<td>R</td>
</tr>
<tr>
<td>SM</td>
<td>500</td>
<td>0.0</td>
<td>108.56(10.30)</td>
<td>3.00(1.28)</td>
<td>0.01</td>
<td>R</td>
</tr>
<tr>
<td>Mon</td>
<td>1000</td>
<td>0.1</td>
<td>20.56(1.23)</td>
<td>3.89(1.89)</td>
<td>0.02</td>
<td>R</td>
</tr>
<tr>
<td>SA</td>
<td>1000</td>
<td>0.0</td>
<td>93.89(1.96)</td>
<td>8.56(2.89)</td>
<td>0.09</td>
<td>R</td>
</tr>
<tr>
<td>SAM</td>
<td>1000</td>
<td>0.0</td>
<td>2.16(1.09)</td>
<td>0.15(0.14)</td>
<td>0.11</td>
<td>R</td>
</tr>
<tr>
<td>SM</td>
<td>1000</td>
<td>0.0</td>
<td>108.44(2.00)</td>
<td>6.33(2.51)</td>
<td>0.00</td>
<td>R</td>
</tr>
<tr>
<td>Mon</td>
<td>5000</td>
<td>0.7</td>
<td>9.39(0.89)</td>
<td>4.33(2.05)</td>
<td>0.00</td>
<td>R</td>
</tr>
<tr>
<td>SA</td>
<td>5000</td>
<td>0.2</td>
<td>90.33(1.94)</td>
<td>5.00(2.22)</td>
<td>0.02</td>
<td>R</td>
</tr>
<tr>
<td>SAM</td>
<td>5000</td>
<td>0.1</td>
<td>13.66(1.31)</td>
<td>6.33(2.50)</td>
<td>0.01</td>
<td>R</td>
</tr>
<tr>
<td>SM</td>
<td>5000</td>
<td>0.0</td>
<td>71.00(1.85)</td>
<td>1.15(0.57)</td>
<td>0.00</td>
<td>R</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>0.38</td>
<td>44.99(2.89)</td>
<td>3.72(1.08)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sam = Solanum lycopersicum “Samrudhi F1”, Mon = Solanum lycopersicum “Mongal F1”, SM = Solanum macrocarpon, and SA = Solanum aethiopicum, R= Resistance, (√x+0)* transformed, R = Rootstock reaction, RF = Reproductive factor.
There were no significant interactive effects between the rootstocks and the various levels of inoculum on fresh root weight, dry root weight and shoot dry weight except the fresh shoot weight twelve weeks after inoculation.

The inoculum level 500 recorded the highest shoot fresh weight (49.92 g) which significantly differed from the shoot fresh weight of the inoculum level 1000 (41.58 g) and the inoculum level of 1000 (44.7 g). Also, the inoculum level 5000 recorded the highest root fresh weight (14.04 g) which significantly differed compared to the inoculum level 500 (7.79 g) (Table 5).

Table 5: Influence of initial inoculum densities of *Meloidogyne* spp. on mean shoot fresh weight, root fresh weight, root dry weight and shoot fresh weight 12 weeks after inoculation.

<table>
<thead>
<tr>
<th>Initial inoculum levels</th>
<th>Shoot fresh weight/g</th>
<th>Root fresh weight/g</th>
<th>Shoot dry weight/g</th>
<th>Root dry weight/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>49.92</td>
<td>7.79</td>
<td>7.00</td>
<td>3.23</td>
</tr>
<tr>
<td>1000</td>
<td>41.58</td>
<td>11.64</td>
<td>7.65</td>
<td>2.76</td>
</tr>
<tr>
<td>5000</td>
<td>44.7</td>
<td>14.04</td>
<td>7.25</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>LSD (P &lt; 0.05)</strong></td>
<td><strong>2.74</strong></td>
<td><strong>2.82</strong></td>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
</tr>
</tbody>
</table>
4.1.2 Detection of Mi gene which confers resistance to root-knot nematodes in Solanum rootstocks. (PCR experiment).

Figure 5: Amplified DNA fragments of Mi gene obtained from four Solanum rootstocks (Lanes:1- *Solanum aethiopicum*, 2- *Solanum lycopersicum* “Samrudhi F1”, 3- *Solanum lycopersicum* “Mongal F1” 4-*Solanum macrocarpon*) and (Lanes: 6-*Solanum aethiopicum* 7-*Solanum lycopersicum* “Samrudhi F1”,8-*Solanum lycopersicum* “Mongal F1” 9-*Solanum macrocarpon*) using Mi gene specific primer pair (PMIF3/R3, C2D1/C2S4) M = Molecular size marker Invitrogen DNA Ladder Mix (1 kb plus); C = Negative controls (sterile distilled water).
The rootstocks *Solanum macrocarpon*, *Solanum aethiopicum* *Solanum lycopersicum* “Samrudhi F1” and *Solanum lycopersicum* “Mongal F1 were screen for the presence of the Mi gene which confers resistance to root-knot nematode infections, the Mi gene specific primers did not amplify the expected Mi gene band sizes in the two rootstocks (*Solanum macrocarpon*, and *Solanum aethiopicum*). On the other hand, the Mi gene specific primers PMIF3/R3 and C2D1/C2S4 amplified two band sizes of 300 and 500bp and a single double band size of 800 bp respectively in the rootstocks *Solanum lycopersicum* “Samrudhi F1” and *Solanum lycopersicum* “Mongal F1. Which were in the region of the expected band sizes of 350 bp and 550 bp for the primer pair PMIF3/R3 and 900 bp for the primer pair C2D1/C2S4 (Figure 5).

**4.2 Fruit quality, shelf life and yield of *Solanum lycopersicum*”Pectomech” on *Solanum macrocarpon*, and *Solanum aethiopicum* in nematode infested soils (Field pot experiment).**

**4.2.1: Grafting success**

The number of grafted plants, graft success and the percentage survival rate as influenced by the grafted rootstock treatments. P/SA and P/SM gave the highest grafting success of 93.8% and 93.3% respectively. P/M gave the lowest survival rate of 0 % (Table 6).

**Table 6: Estimated grafting success of rootstocks.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of grafted plants</th>
<th>Graft Success</th>
<th>Percentage graft success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/M</td>
<td>196</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P/SM</td>
<td>196</td>
<td>183</td>
<td>93.3</td>
</tr>
<tr>
<td>P/SA</td>
<td>196</td>
<td>184</td>
<td>93.8</td>
</tr>
</tbody>
</table>

P/M = Pectomech grafted onto *Solanum lycopersicon*”Mongal F1”, P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted onto *Solanum macrocarpon*. 
4.2.2 General observations of grafted plants.

Prominent rootstock regrowth was noticed thirty days after initial cut back before transplanting. Both rootstocks exhibited vigorous regrowth which occurred beneath the graft union on the rootstock, which was also an indication of how vigorous the rootstocks were (Figure 6).

![Figure 6: Adventitious bud regrowth. A= Solanum macrocarpon rootstock with bud regrowth. B = Solanum aethiopicum rootstock with bud regrowth.](image)

The grafting rootstocks, *Solanum macrocarpon* and *Solanum aethiopicum* rootstocks under varying root knot nematode inoculum (500, 1000 and 5000) did not show any extensive root knot nematode damage after fruit harvest.
Figure 7: Fresh root biomass of grafted *Solanum* spp. roots under varying nematode density. SA500 = *Solanum aethiopicum* rootstock + 500 nematodes, SA1000 = *Solanum aethiopicum* rootstock + 1000 nematodes, SA5000 = *Solanum aethiopicum* rootstock + 1000 nematodes. SM500 = *Solanum aethiopicum* rootstock + 500 nematodes, SM1000 = *Solanum aethiopicum* rootstock + 1000 nematodes, SM5000 = *Solanum aethiopicum* rootstock + 1000 nematodes.
There were no significant interactive effects between the varying density of nematode inoculums and grafted rootstocks on chlorophyll content.

Similarly, there were also no significant differences among varying density of nematode inoculums on chlorophyll content.

There was no significant interactive effect between the varying inoculum density of *Meloidogyne* spp. and grafted rootstocks on plant height.

There were significant differences in plant height 56 and 70 days after inoculation. Plant height after 56 days decrease with increasing inoculum density from 0, 500 to 1000 (35.48 cm, 33.87 cm and 30.42 cm) similarly plant height decreased with increasing inoculum after 70 days the inoculum level 5000 recorded the highest plant height (34.24 cm and 40.9 cm) after 56 and 70 days respectively. On the other hand, the inoculum density 1000 recorded the least plant height (30.42 cm and 36.8 cm) after 56 and 70 days respectively (Table 7).

**Table 7: Influence of inoculum density of *Meloidogyne* spp. on plant height (cm).**

<table>
<thead>
<tr>
<th>Nematode inoculum density</th>
<th>14</th>
<th>28</th>
<th>42</th>
<th>56</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.42</td>
<td>20.93</td>
<td>25.24</td>
<td>35.48</td>
<td>41.79</td>
</tr>
<tr>
<td>500</td>
<td>13.66</td>
<td>20.71</td>
<td>25.08</td>
<td>33.87</td>
<td>40.57</td>
</tr>
<tr>
<td>1000</td>
<td>13.65</td>
<td>18.68</td>
<td>24.18</td>
<td>30.42</td>
<td>36.80</td>
</tr>
<tr>
<td>5000</td>
<td>15.08</td>
<td>20.95</td>
<td>25.58</td>
<td>34.24</td>
<td>40.9</td>
</tr>
<tr>
<td><strong>LSD (P &lt; 0.05)</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td><strong>3.25</strong></td>
<td><strong>2.88</strong></td>
</tr>
</tbody>
</table>
There were no significant interactive effects between the varying densities of nematode inoculum and grafted rootstocks on plant girth.

The inoculum density 500 recorded the highest plant girth (4.90) which significantly differed from the plant girth recorded by the inoculum density 1000 which recorded the least plant girth (4.14) (Table 8).

**Table 8: Influence of inoculum density of *Meloidogyne* spp. on plant girth (mm).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after inoculation</th>
<th>14</th>
<th>28</th>
<th>42</th>
<th>56</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>4.76</td>
<td>5.66</td>
<td>6.31</td>
<td>7.20</td>
<td>7.61</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>4.90</td>
<td>7.50</td>
<td>6.60</td>
<td>7.68</td>
<td>8.02</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>4.15</td>
<td>5.33</td>
<td>6.19</td>
<td>7.46</td>
<td>8.29</td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>4.62</td>
<td>5.37</td>
<td>6.54</td>
<td>7.90</td>
<td>8.35</td>
</tr>
</tbody>
</table>

LSD (P < 0.05) | 0.59 | NS | NS | NS | NS
There were no significant interactive effects between the varying density of nematode inoculums and grafted rootstocks on the days to fifty percent flowering, number of fruits per plant and yield per plant.

The inoculum density with no *Meloidogyne* spp. recorded the highest yield per plant in grams (56.3 g) which was significantly higher than the inoculum density 5000 which recorded the lowest yield per plant in grams (36.2 g) Table 9.

**Table 9: Influence of inoculum density of *Meloidogyne* spp. on DFF (%), number of fruits per plant and yield/plant (g)**

<table>
<thead>
<tr>
<th>Inoculum density</th>
<th>DFF (%)</th>
<th>NFRTS</th>
<th>Yield/plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31.67</td>
<td>6.83</td>
<td>56.3</td>
</tr>
<tr>
<td>500</td>
<td>33.17</td>
<td>6.30</td>
<td>46.2</td>
</tr>
<tr>
<td>1000</td>
<td>30.67</td>
<td>6.73</td>
<td>45.1</td>
</tr>
<tr>
<td>5000</td>
<td>32.67</td>
<td>5.97</td>
<td>36.2</td>
</tr>
<tr>
<td><strong>LSD (P &lt; 0.05)</strong></td>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
<td><strong>14.62</strong></td>
</tr>
</tbody>
</table>

There were no significant interactions between the grafted *Solanum* rootstocks and the inoculum density on TSS, TSS/TA, pH and TA.

There were no significant differences in fruit shelf life 15, 20, 25, 30 and 40 days after harvest, however, there were significant differences in inoculum and grafted treatments on shelf life 35 days after harvest. The combination effect between the grafted rootstocks P/SA and P/SM with the varying inoculum levels 500 and 5000 recorded the highest fruit loss (73.3%) after 35 days which was significantly higher (P < 0.05) compared to the control rootstocks P/SA and P/SM with fruit loss of 33.3% and 46.7% respectively (Table 10).
Table 10: Influence of inoculum density of *Meloidogyne* spp. and grafted *Solanum* spp. on fruit shelf life.

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>Inoculum density</th>
<th>15 DAYS</th>
<th>20 DAYS</th>
<th>25 DAYS</th>
<th>30 DAYS</th>
<th>35 DAYS</th>
<th>40 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/SA</td>
<td>500</td>
<td>13.3</td>
<td>20</td>
<td>33.3</td>
<td>33.3</td>
<td>60</td>
<td>86.7</td>
</tr>
<tr>
<td>P/SM</td>
<td>500</td>
<td>6.7</td>
<td>13.3</td>
<td>20</td>
<td>46.7</td>
<td>73.3</td>
<td>73.3</td>
</tr>
<tr>
<td>P/SA</td>
<td>1000</td>
<td>0.0</td>
<td>20</td>
<td>20</td>
<td>26.7</td>
<td>46.7</td>
<td>73.3</td>
</tr>
<tr>
<td>P/SM</td>
<td>1000</td>
<td>6.7</td>
<td>6.7</td>
<td>13.3</td>
<td>40</td>
<td>66.7</td>
<td>86.7</td>
</tr>
<tr>
<td>P/SA</td>
<td>5000</td>
<td>6.7</td>
<td>26.7</td>
<td>26.7</td>
<td>40</td>
<td>73.3</td>
<td>86.7</td>
</tr>
<tr>
<td>P/SM</td>
<td>5000</td>
<td>13.3</td>
<td>13.3</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>53.3</td>
</tr>
</tbody>
</table>

LSD (P < 0.05) | NS | NS | NS | NS | 14.91 | NS

P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted onto *Solanum macrocarpon*.

There were no significant interactive effects between grafted rootstocks and varying inoculum density on root fresh weight, root dry weight, shoot fresh weight, shoot dry weight (g).

The inoculum density 1000 recorded the highest root dry weight (25.4 g) which was significantly higher compared to the dry root weight of the inoculum density 5000 (10.7 g) which recorded the least root dry weight (Table 11).
Table 11: Influence of inoculum density of *Meloidogyne* spp. on root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight (g).

<table>
<thead>
<tr>
<th>Inoculum density</th>
<th>RFWg</th>
<th>RDWg</th>
<th>SFWg</th>
<th>SDWg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28.2</td>
<td>12.3</td>
<td>24.2</td>
<td>14.30</td>
</tr>
<tr>
<td>500</td>
<td>28.0</td>
<td>19.2</td>
<td>19.6</td>
<td>12.09</td>
</tr>
<tr>
<td>1000</td>
<td>34.5</td>
<td>25.4</td>
<td>23.3</td>
<td>13.35</td>
</tr>
<tr>
<td>5000</td>
<td>30.2</td>
<td>10.7</td>
<td>23.1</td>
<td>14.78</td>
</tr>
</tbody>
</table>

LSD (P < 0.05) NS 7.36 NS NS

RFW= root fresh weight, RDW= root dry weight, SFW= shoot dry weight, SDW= shoot dry weight, LSD = Least significant difference.

4.3 Fruit quality, shelf life and yield of *Solanum lycopersicum* “Pectomech” grafted onto *Solanum macrocarpon* and *Solanum aethiopicum* in open field root knot nematode infested soils

4.3.1: Grafting success

The grafted rootstocks P/SA and P/SM gave the highest grafting success of 94% whilst P/M gave the lowest survival rate of 1% (Table 12).

Table 12: Estimated grafting success of rootstocks.

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>Number of grafted Plants</th>
<th>Graft Success</th>
<th>Percentage Graft Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/M</td>
<td>196</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>P/SM</td>
<td>196</td>
<td>184</td>
<td>94</td>
</tr>
<tr>
<td>P/SA</td>
<td>196</td>
<td>185</td>
<td>94</td>
</tr>
</tbody>
</table>

P/M = Pectomech grafted on *Solanum lycopersicon “Mongal F1”*, P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted *Solanum macrocarpon*. 
4.3.2 General observations made on grafted plants.

Rootstock regrowth which is also called “suckering” or adventitious bud growth was observed fourteen (days) after achieving grafting success. The regrowth was vigorous and occurred beneath the graft union on the rootstock. Both rootstocks (*Solanum macrocarpon* and *Solanum aethiopicum*) used in this experiment exhibited adventitious bud regrowth (Figure 8).

![Figure 8: Grafted tomato plants exhibiting adventitious bud regrowth.](image)

An out-break of fusarium wilt was also observed in each of the three replicates which resulted in the loss of plants however, the attack was only limited to the non-grafted plants which served as a control in each of the three replicates. A symptom of the disease was first observed one and a half months after transplanting. Eleven plants out of the total twenty-four non-grafted recording plants were infected (Table 13).
Table 13: Estimated disease intensity of plants infected by Fusarium wilt.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NRP</th>
<th>NDRP</th>
<th>DI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>11</td>
<td>45.8</td>
</tr>
<tr>
<td>P/SM</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P/SA</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NRP = Number of recording plants, NDRP = Number of diseased recorded plants, DI = Disease intensity (%), P/SA = Pectomech grafted onto Solanum aethiopicum, P/SM = Pectomech grafted Solanum macrocarpon.

A cross-section of the stem of sampled infected plants showed browning of the xylem tissues or the vascular tissues with brown streaks along the length of the stem. Cultured tissues of the sampled plants on a PDA also revealed a subtle whitish to pinkish growth with a purple trace (Figure 9).
Figure 9: Symptoms of fusarium wilt disease of tomato. A- Early symptoms with isolated number of yellow leaves; B- Advance symptoms with many yellow leaves; C- Browning and wilting of leaves; D - Browning of the vascular tissues; E- Cultured stem tissues of infected plant on potato dextrose agar.
Fruits harvested from Pectomech grafted onto *Solanum aethiopicum* rootstocks, Pectomech grafted *Solanum macrocarpon* and ungrafted Pectomech were comparable to each other.

**Figure 10:** Tomato fruits from pectomech variety grafted on *Solanum* spp. rootstocks. P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted *Solanum macrocarpon*.

There were no significant differences among the grafted and non-grafted Solanum spp. rootstocks regarding plant height at 14, 28, 42 and 56 days after transplanting.

The grafted Solanum spp. rootstocks P/SA and P/SM significantly increased chlorophyll content from 14 days after transplanting to 28 days after transplanting. The rootstock P/SA recorded the highest chlorophyll content (51.2 and 62.3) 14 and 28 days after transplanting. However, there were no significant differences (P < 0.05) between treatments in chlorophyll content from 42 days after transplanting to 56 days after transplanting (Table 14).
Table 14: Influence of grafted *Solanum* spp. rootstocks on chlorophyll content.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>14 DAT</th>
<th>28 DAT</th>
<th>42 DAT</th>
<th>56 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>40.0</td>
<td>29.1</td>
<td>22.4</td>
<td>16.52</td>
</tr>
<tr>
<td>P/SA</td>
<td>51.2</td>
<td>62.3</td>
<td>24.1</td>
<td>12.7</td>
</tr>
<tr>
<td>P/SM</td>
<td>55.8</td>
<td>57.5</td>
<td>30.9</td>
<td>18.7</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>7.9</td>
<td><strong>20.92</strong></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

DAT = Days after transplanting, Control = non-grafted tomato plant (Pectomech), P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted onto *Solanum macrocarpon*, LSD = Least significant difference.

There were no significant differences (P < 0.05) in plant girth among the grafted and non-grafted *Solanum* spp. rootstocks from 14 days to 42 days after transplanting. However, there were significant differences after 56 days. The grafted rootstocks P/SA recorded the highest plant girth (7.16) which was significantly higher compared to the non-grafted rootstocks (5.17) Table 15.

Table 15: Influence of grafted *Solanum* spp. rootstocks on plant girth (mm)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>14 DAT</th>
<th>28 DAT</th>
<th>42 DAT</th>
<th>56 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>4.64</td>
<td>7.81</td>
<td>7.04</td>
<td>5.17</td>
</tr>
<tr>
<td>P/SA</td>
<td>7.63</td>
<td>9.52</td>
<td>7.75</td>
<td>7.16</td>
</tr>
<tr>
<td>P/SM</td>
<td>5.75</td>
<td>7.24</td>
<td>6.41</td>
<td>6.0</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td><strong>1.3</strong></td>
</tr>
</tbody>
</table>

DAT = Days after transplanting, Control = non-grafted tomato plant (Pectomech), P/SA = Pectomech grafted onto *Solanum aethiopicum*, P/SM = Pectomech grafted onto *Solanum macrocarpon*, LSD = Least significant difference.
The grafted rootstocks P/SA and P/SM significantly increased the yield per plant and number of fruits per plant. The grafted rootstock P/SA recorded the highest yield and number of fruits per plant (461g and 17) which was significantly higher compared to the control or the ungrafted pectomech which recorded the least yield and number of fruits per plant (152 g and 6). However, there were no significant differences in fruit diameter and days to 50% flowering (Table 16).

Table 16: Influence of grafted Solanum spp. rootstocks on yield per plant, number of fruits per plant, fruit diameter and days to 50% flowering.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield per plant/g</th>
<th>NFRTS/plant</th>
<th>FD/mm</th>
<th>DFF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>152</td>
<td>6.0</td>
<td>35.0</td>
<td>29.3</td>
</tr>
<tr>
<td>P/SA</td>
<td>461</td>
<td>17.0</td>
<td>33.4</td>
<td>33.3</td>
</tr>
<tr>
<td>P/SM</td>
<td>224</td>
<td>13.0</td>
<td>32.1</td>
<td>31.0</td>
</tr>
</tbody>
</table>

LSD (P < 0.05) 201.2 2.2 NS NS

NFRTS = Number of fruits, Control = non-grafted tomato plant (Pectomech), FD = Fruit diameter, DFF = days to fifty percent flowering, P/SA = Pectomech grafted onto Solanum aethiopicum, P/SM = Pectomech grafted onto Solanum macrocarpon, LSD = Least significant difference.

There were no significant differences in mean Total soluble solids, Titrable acidity, and Total soluble solids to titrable acidity ratio and pH between grafted and non-grafted Solanum spp. rootstocks.

There were significant differences in shoot fresh and shoot dry weight between the grafted and non-grafted treatments, the rootstock P/SA recorded the highest shoot fresh weight (11.40 g) and shoot dry weight (42.1g) was significantly higher compared to the control (18.7g) or the ungrafted rootstock of 7.62 g and 18.7 g shoot fresh weight and dry weight respectively. Also the grafted rootstock P/SM recorded the lowest shoot fresh weight and dry weight of (6.88 g and 17 g) which was significantly lower compared to the shoot fresh and dry weight of the rootstock.
P/SA. Also, there were no significant differences in root dry weight and fresh weight for the treatments studied (Table 17).

**Table 17: Influence of grafted Solanum spp. rootstocks on root fresh weight, root dry weight, shoot fresh weight and shoot dry weight.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RFWg</th>
<th>RDWg</th>
<th>SFWg</th>
<th>SDWg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>4.3</td>
<td>1.85</td>
<td>59 (7.62) *</td>
<td>18.7</td>
</tr>
<tr>
<td>P/SA</td>
<td>10.4</td>
<td>3.32</td>
<td>140 (11.40) *</td>
<td>42.1</td>
</tr>
<tr>
<td>P/SM</td>
<td>5.8</td>
<td>3.03</td>
<td>51 (6.88) *</td>
<td>17.0</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>NS</td>
<td>NS</td>
<td>104(4.1) *</td>
<td>14</td>
</tr>
</tbody>
</table>

Control = Non-grafted tomato plant (Pectomech), P/SA = Pectomech grafted onto Solanum aethiopicum, P/SM = Pectomech grafted onto Solanum macrocarpon. RFW= root fresh weight, RDW= root dry weight, SFW= shoot dry weight, SDW= shoot dry weight, LSD = Least significant difference. ( )* = transformed data.

There were no significant differences in the shelf life of the fruits of grafted and non-grafted Solanum spp. rootstocks.
CHAPTER 5

5.0 DISCUSSION

5.1 Screening of Solanum spp. rootstocks against root-knot nematodes

Six weeks after inoculation of the Solanum spp. with root knot nematodes the interaction between the rootstocks and the initial nematode population density led to a reduction in the final nematode populations with respect to their reproduction factors (RF’s). There were also no interactions between the rootstocks and the inoculum density on the mean gall scores, egg count per gram of roots and nematode count per 200 cc of soil. The interactive effect between the rootstocks Solanum macrocarpon and Solanum lycopersicum “Mongal F1” and the inoculum density 5000 also recorded the least reproductive factor of 0.02.

An increase in the main inoculum density effect from 500 to 1000 significantly increased the gall scores from 0.28 to 0.68. Interestingly, there were no significant increases in nematode count per 200 cc of soil (juvenile population). According to the findings of Jaiteh et al. (2012) where 33 tomato genotypes were screened to evaluate the reaction to root-knot nematode under five inoculum levels (100, 500, 1000, 1500 and 2000), increasing inoculum density resulted a corresponding increase in gall score which was supported by the findings of this study. Also with an increase in initial inoculum density from 100 to 1500 there was a direct increase in the population of juvenile nematodes which was not supported by the findings of this study. However, among the 33 tomato genotypes tested Solanum lycopersicum “Mongal T-11” and tomato Beef Master were identified as resistant rootstocks because their reproductive factor was 0.71 and 0.53 which was less than one (<1), and was similar to the findings of this study. Karssen and Moens (2006), explained that a rootstock is resistant to root knot nematodes if it
able to prevent the development of the organisms within its root and thus preventing it from reproducing. The rootstocks studied after six weeks were therefore resistant to root knot nematodes since their reproductive factors were less than one.

5.1.2 Fresh root weight, dry root weight, fresh shoot weight and dry root weight of rootstocks 6 weeks after inoculation.

The interaction effect between the rootstocks and the initial inoculum density did not have any effect on the fresh root weight, dry root weight, fresh shoot weight and dry root weight six weeks after inoculation. The shoot fresh weight significantly decreased from 51.8 g to 36.9 g with the main inoculum treatment effect from 500 to 1000. In a research conducted to investigate the influence of *Meloidogyne incognita* density of 50, 500, 1000, 1500 eggs on susceptible tomato by Kamran et al. (2013), a significant decrease in shoot fresh weight with increasing nematode density from 34.5g, 33.13g, 26.35g and 22.83g respectively in was revealed which corroborates the findings of this study.

5.1.3 Gall scores, egg count per gram of roots, nematode count per 200 cc of soil and reproductive factor of rootstocks 12 weeks after inoculation.

Twelve weeks after inoculation the interactive effect between the rootstocks and the initial inoculum density reduced the mean gall score and the reproductive factor with *Solanum lycopersicum* “Samrudhi F1”, *Solanum lycopersicum* “Samrudhi F1” and *Solanum lycopersicum* “Mongal F1” and the inoculum levels 500, 1000 and 5000 yielding the least nematode count per 200 cc of soil of 1.63, 1.09 and 0.89 respectively. Investigating the susceptibility of seven varieties of pepper and tomato to root knot nematodes and the usage of both the galling index their reproductive factors interpreted as (GI ≤ 2, RF ≤ 1) = resistant, (GI ≥ 2, RF > 1) = susceptible, (GI ≤ 2, RF > 1) = tolerant, Bello et al. (2015), reports that all the tomato genotypes
screened, Big beef, Celebrity, Jetsetter, Roma VFN, and Smalflly varieties of tomato were rated tolerant with GI ≤ 2 and RF > 1 which is contrary to findings of this experiment since all the rootstocks screened recorded GI ≤ 2, RF ≤ 1 after 12 weeks.

5.1.4 Fresh root weight, dry root weight, fresh shoot weight and dry root weight of rootstocks 12 weeks after inoculation

The combination effect between the rootstocks and the initial inoculum density did not have any effect on the fresh root weight, dry root weight, fresh shoot weight and dry root weight twelve weeks after inoculation. The shoot fresh weight significantly decreased from 49.92 g to 41.58 g with the main inoculum density effect from 500 to 1000. The root fresh weight significantly increased (7.9 g and 14.04 g) with increasing inoculum density from 500 to 1000 respectively. The results of this study also agreed with the findings of Kamran et al. (2013) which revealed a significant decrease (P<0.05) in shoot fresh weight with increasing nematode density (50, 500, 1000, 1500 eggs) and 34.5 g, 33.13 g, 26.35 g and 22.83 g shoot fresh weight respectively.

5.1.5 Screening Solanum macrocarpon, Solanum aethiopicum Solanum lycopersicum “Samrudhi F1”, and Solanum lycopersicum “Mongal F1 for presence of Mi gene which (confers resistance to root-knot nematode)

The rootstocks Solanum lycopersicum “Samrudhi F1”, and Solanum lycopersicum “Mongal F1 amplified a single band of 800bp, and a double band of 300 and 500bp which confirmed the presence of the Mi gene in these rootstocks using the gene specific primers pairs of C2D1/C2S4 and PMiF3/PMiR3; On the other hand the rootstocks Solanum macrocarpon and Solanum aethiopicum did not produce any bands which also indicates that they do not possess the Mi gene, the results obtained was in line with the work done by Goggin et al. (2006), in their work they reintroduced the Mi gene into Solanum melongena to assess the performance of the gene in
conferring resistance to rootknot nematode *Meloidogyne javanica* and aphids, in confirming the presence of the gene in the eggplant a single band of 915bp was amplified. Similarly the work done by Mehrach *et al.* (2007), also confirms the results obtained in this study, their objective was to detect the presence of the Mi-1.2 gene which confers resistance to rootknot nematodes in 14 begomovirus-resistant breeding lines with known resistant and susceptible cultivars in a two steps PCR method, using the primer pairs PMiF3/PMiR3 a single unique band of 350bp was produced for susceptible cultivars and a double band of 350bp and 550bp was produced for resistant cultivars.

5.2 Fruit quality, shelf life and yield of *Solanum lycopersicum”Pectomech”* on *Solanum macrocarpon, and Solanum aethiopicum* in nematode infested soils (Field pot experiment).

The results in this study showed interaction effect of grafted *Solanum lycopersicum” Pectomech”* and varying nematode inoculum on fruit yield, fruit quality and shelf life.

5.2.1 Grafting success

Grafting success in *Solanum macrocarpon, and Solanum aethiopicum* rootstocks using the cleft graft method gave high grafting percentage of 93.3% and 93.8% respectively. These results are similar to the findings of Maršić and Osvald (2004), who reported that grafting of two tomato cultivars (“PG3” and “Beaufort”) using the clef method resulted in a high percentage graft rate of 79-100% indicating the suitability of the grafting method for tomato grafting. However, the low grafting percentage recorded by Pectomech grafted on *Solanum lycopersicon”Mongal F1”* rootstock was not consistent with their findings.
5.2.2 Adventitious bud regrowth in grafted rootstocks

The rootstocks *Solanum macrocarpon*, and *Solanum aethiopicum* exhibited adventitious bud growth which is a common observation in most grafted plants. The growth was observed 30 days after transplanting. This observation was also made by Bausher (2011), who reported that most vigorous rootstocks tend to exhibit bud regrowth in grafted plants.

5.2.3: Effect of varying nematode density on root biomass of grafted rootstocks

Generally galling of grafted rootstock were greatly reduced under the various density of root knot nematode inoculums. This observation was similar to the findings of Barrett *et al.* (2012) who reported that when susceptible heirloom tomato cultivars (*S. lycopersicum* “Brandywine” and *S. lycopersicum* “Flamme”) were grafted onto two hybrid rootstocks (*S. lycopersicum* “Multifort” and *S. lycopersicum* “Survivor”) with non-grafted and self-grafted as controls, root damage or galling was significantly reduced by 81 percent.

5.2.4 Chlorophyll content, plant height and plant girth of grafted rootstocks

There were no significant interactive effects between the inoculum density and grafted rootstocks and the main inoculum treatment effect on chlorophyll content of the grafted rootstocks. Equally plant height and girth were also not affected by the treatment combination. In an experiment to investigate changes in leaf photosynthetic capacity of a hybrid eggplant *Solanum melongena* used as rootstock to control root knot nematodes in Greece, grafting did not enhance the photosynthetic capabilities of the leaves of the scion (Khah *et al.* 2006). This was in line with the findings of this study. The main inoculum treatment effect had an inverse effect on plant height with increasing inoculum levels 56 days after inoculation however; increasing inoculum density did not have an increasing effect on plant girth. In investigating the effect of inoculum densities of root knot nematodes on the growth of tomato cv. Kamkam and Adomako, (2014), reported
that all inoculum densities reduced the plant height which agreed with the findings of this study but not in terms of plant girth. In explaining the relationship between plant heights and increasing inoculum density, it is possible that with increasing inoculum levels, higher number of nematode juveniles gain entry into the root system of the plant thereby reducing the amount of nutrients which is made available to the plant for plant growth through their intense feeding activities.

5.2.5 Days to 50% flowering, number of fruits and yield per plant

The combination effect between the grafted rootstock and inoculum density did not have any effect on Days to 50% flowering and fruit yield per plant, however, increase in the main inoculum level had a decreasing effect on fruit yield per plant. The inoculum level 5000 recorded the least plant yield per plant (36.2g) whilst the inoculum level recorded the highest yield per plant (46.2 g) without the control. A pathogenicity study carried out on southern root-knot nematode on Roma king tomato cultivar under the inoculum levels 2000, 4, 000, 6, 000, 8, 000 and 10, 000 by Bawa et al. (2014), revealed that a progressive increase in the inoculum levels had an increasing effect on fruit yield and the number of fruits per plant. In addition, at the inoculum level of 8,000 there was a 100% loss in fruit weight per plant at a higher inoculum level of 8,000 eggs. These findings corroborate the findings of this study. There is a possibility that with increasing nematode inoculum the number of nematode feeding on the root per plant increases leading to a reduction in plant nutrients which eventually culminates into the reduction in yields as observed.

5.2.6 Titrable acidity, Total soluble solids, and pH

There was no significant interactive effect between the varying levels of nematode inoculums and grafted rootstocks as well as the main inoculum density effect on titrable acidity, total
soluble solids, and pH of the grafted rootstocks. Similar findings were made by Barrett et al. (2012) where rootstocks do not alter the fruit quality attributes of tomato scions.

5.2.7 Root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight

There were no significant interactive effects between the varying levels of nematode inoculums and grafted rootstocks on root fresh weight, root dry weight, shoot fresh weight, shoot dry weight. The inoculum level 1000 recorded the highest root dry weight (25.4g) which was significantly higher compared to the dry root weight of the inoculum level 5000 (10.7g) which recorded the least root dry weight. The findings of this study however contradict that of Kankam and Adomako (2014), who reported that increasing nematode inoculum levels does have an effect on fresh and dry root weights.

5.3 Fruit quality, shelf life and yield of Solanum lycopersicum "Pectomech" on Solanum macrocarpon, and Solanum aethiopicum in nematode infested soils (Open field experiment)

This study was conducted to determine the yield, fruit quality, and shelf life of Solanum lycopersicum "Pectomech" grafted onto Solanum macrocarpon and Solanum aethiopicum under open field conditions.

5.3.1 Grafting success

Using the cleft graft method, the grafting rootstocks P/SA and P/SM gave the highest grafting success of 94%, on the other hand the grafted rootstock P/M recorded the least grafting success of 1% due to pathogen contamination. The use of cleft grafting method ensured a high grafting percentage since there is high tissue surface contact between the scion and the rootstock. Using the cleft grafting method Maršić and Osvald, (2004) reported that graft success above 80% is normally achieved.
5.3.2 Observations

Fourteen days after grafting the rootstocks (*Solanum aethiopicum* and *Solanum macrocarpon*) developed adventitious bud regrowth, even though such a growth indicates that the rootstock is vigorous Guan, (2011), explains that it is a major concern which if not well managed can lead to an increase in labor cost.

Also, six weeks after transplanting to the field there was an outbreak of *fusarium* wilt which attacked the non-grafted plants in the field since the fungi *Fusarium oxysporum* f. sp. *Lycopersici* is host specific to tomato. The pathogen can live in the soil and the living tissues of its host and mostly occurs during fruiting of the plant (Wong, 2003).

Finally, the fruits of the various grafted and non-grafted rootstocks were comparable in size and there were no clear-cut differences an indication that grafting gave no added advantage in terms of the fruit parameters studied.

5.3.3 Plant height, chlorophyll content and plant girth

There were no significant differences in plant height between the grafted and non-grafted rootstocks 56 days after transplanting. The rootstock P/SA recorded the highest chlorophyll content 14 and 28 days after transplanting which differed significantly compared to the control or non-grafted rootstocks. There were however, no significant differences between the grafted rootstocks P/SA and P/SM during the same period. Also, the grafted rootstock P/SA recorded the highest scion girth 56 days after transplanting which was significantly higher compared to the non-grafted rootstocks. In evaluating the variation of plant growth and macronutrient uptake in grafted tomato ('Rita' Fi) and eggplant ('Mission Bell' Fi) grafted onto three rootstocks Energy, PG3 and Beaufort. Leonardi and Giuffrida, (2006) reported that tomato ('Rita' Fi) grafted on Beaufort gave a greater plant height compared to the control which was self-grafted a claim
which was contrary to the findings of this study. However, there were no significant differences in plant girth when the tomato ('Rita' F1) was grafted on Beaufort compared to the self-graft or control another claim which was contrary to the findings of this study. Finally, in another experiment to determine the growth and yield of tomato (Cecilia F1) on three different rootstocks (Beaufort, He-man and a local cultivar), Mohammed et al. (2009), reported that chlorophyll content was significantly increased in Cecilia grafted onto Beaufort as well as Cecilia grafted onto He-man compared to the non-grafted Cecilia which agreed with the findings of this study.

5.3.4 Yield per plant, number of fruits per plant, fruit diameter and days to 50% flowering

Yield per plant in grams and numbers of fruits per plant in this study were found to be significantly higher in P/SA compared to the non-grafted control, however fruit diameter and days to 50% flowering were not significantly different. According to Maršić and Osvald, (2004) when the influence of grafting on the success of Monroe' and 'Belle grafted on 'PG 3' and 'Beaufort' respectively were determine in a study the scion and rootstock combination between Monroe and Beaufort significantly increased the total yield and the number of fruits per plant compared to the non-grafted plant which agrees with the findings of this study. Mohammed et al. (2009), also reported that when Cecilia (scion) was grafted onto Beaufort the fruit size was not significantly different from the control which agreed with the findings of this study. According to Khah et al. (2006), in an experiment were the tomato cultivars Big Red’ was used as scion with ‘Heman’ and ‘Primavera’ as rootstocks in greenhouse and open field cultivation, it was observed that the and non-grafted controls (Big Red) flowered earlier compared to the grafted plants. This he explained could be a result of the stress imposed on the plant by grafting, however, his finding was not consisted with the findings of this study.
5.4.5 Total soluble solids, Titrable acidity, and pH

There were no significant differences among the fruit quality attributes measured. These results were also confirmed by Khah et al. (2006) where they compared the fruit quality attributes of the tomato cultivars Big Red’ grafted on ‘Heman’ and ‘Primavera’ in both open field and greenhouse. They reported that there were no significant differences in total soluble solids, titrable acidity, pH and lycopene content among the grafted and non-grafted controls. This is evidence that fruit quality attributes are scion specific and not influenced by the rootstocks, generally rootstocks confer resistance to pathogens infestations to the scion, and also this observed trend could be due to the fact that the grafted and non-grafted rootstocks received the same levels of treatments such as fertilizer applications.

5.4.5 Root fresh weight, root dry weight, shoot fresh weight and shoot dry weight

There were no significant differences in root fresh and dry weight, however, there were significant differences in shoot fresh and dry weight, the grafted rootstock P/SA recorded the highest shoot fresh and dry weight which was significantly different compared to the control or the non-grafted as well as the grafted rootstock P/SM. In reference to the work done by Mohammed et al. (2009) where the tomato cultivar Cecillia F1 was grafted onto Beaufort and He-man rootstocks the root fresh and dry weight were found to be significantly higher compared to the non-grafted Cecillia genotype which was not consistent with the findings of this study, equally the shoot and fresh weight of the grafted rootstocks were significantly higher compared to the control.

5.4.5 Shelf life of grafted and non-grafted tomato fruits

The shelf life which is the duration or the time taken for stored tomato fruits to go bad was found not to be significantly different among the grafted and non-grafted treatment. This finding was
found to be in line with the findings by Qaryouti et al. (2007). In their research where they studied the effect of grafting on yield, fruit quality and shelf life of tomato the tomato cultivar “Cecilia F1” grafted on to “He-man” and “Spirit” using simplified open soilless system vs. conventional soil cultivation, they reported that even though the fruit quality attributes such as the Vitamin C and total soluble solids content were improved the shelf life was not affected in the two growth media.
CHAPTER SIX

6.0 CONCLUSION

- The interaction effect of rootstocks and varying inoculum density of *Meloidogyne* spp. reduced the level of root damage and the reproductive factor of the rootstocks (*Solanum macrocarpon* and *Solanum aethiopicum*). However, using Mi gene specific primers (PMIR3/F3 and C2D1/C2S4) revealed that the rootstocks *Solanum lycopersicum* “Samrudhi” and *Solanum lycopersicum* “Mongal” possessed the Mi gene with double PCR band sizes of 300 bp and 500 bp and a single band size of 800 bp for the primer pair of PMIR3/F3 and C2D1/C2S4 respectively whilst *Solanum macrocarpon* and *Solanum aethiopicum* bands did not possess the Mi gene. This is an indication that the rootstocks (*Solanum macrocarpon* and *Solanum aethiopicum*) are tolerant to root-knot nematode infestations which may be as a result of the possession of other resistance mechanisms other than Mi genes.

- The interaction effect of increasing inoculum density of *Meloidogyne* spp. and grafted *Solanum* spp. rootstocks (*Solanum macrocarpon* and *Solanum aethiopicum*) showed no significant influence on yield, shelf life and fruit quality attributes of grafted *Solanum lycopersicum* “Pectomech” fruits.

- Grafting success of *Solanum lycopersicum* “Pectomech” on *Solanum* spp. rootstocks (*Solanum macrocarpon* and *Solanum aethiopicum*) recorded 94% success respectively. The grafted *Solanum* spp. rootstock (*Solanum aethiopicum*) recorded significant increase in yield of fruits per plant (461g), however, the grafting *Solanum* rootstocks showed no significant influence on fruit quality and shelf life a possible indication that the function of a rootstock in a graft combination is more focused on vigorous growth.
and tolerance of soil borne diseases, while fruit attributes are a result of the scion’s characteristics.
RECOMMENDATIONS

- Further studies should be conducted on *Solanum macrocarpon* and *Solanum aethiopicum* to determine root knot nematode resistance mechanisms other than the possession of Mi genes.

- Rootstocks of other indigenous *Solanum* spp. should be screened for resistance against root knot nematode and evaluated for graft compatibility.
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