CHARACTERISATION OF CHILLI ROOT KNOT NEMATODES (*Meloidogyne* spp.) AND EVALUATION OF SODOM APPLE (*Solanum incanum* L.) PLANT EXTRACT AND *Trichoderma viride* C. AS CONTROL AGENTS IN NAKURU COUNTY, KENYA

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A Thesis submitted to the Graduate School in Partial fulfillment for the Requirements of the award of Master of Science Degree in Plant Pathology of Egerton University.

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for a degree in any university

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Recommendation

This thesis has been submitted to the Board of Postgraduate studies for examination with our approval as University supervisors as per the Egerton University regulations.

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DEDICATION

To my dear wife Naomi Njoki, sisters Susan, Judy, Evalyne, nephews Jayden, Ryan and Jeremy.

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ABSTRACT

Chilli (*Capsicum annum* L.**)** is a tropical and sub-tropical crop grown for its pungent tasting fruits and sour leaves. Chillis are prone to root knot nematodes (*Meloidogyne* spp.) which reduce their quality and yields. The study focused on characterisation of root knot nematodes infesting chilli in Nakuru County, investigation on the use of sodom apple fruit extracts in controlling root knot nematodes, the use of *Trichoderma viride* as a potential bio-control agent against root knot nematodes and evaluation of the effect of root knot nematodes on the uptake of nitrogen and phosphorous in chilli when sodom apple fruit and *Trichoderma viride* extracts were applied. Infested root samples were drawn randomly from 7 sub-counties of Nakuru County. Three farms were sampled per sub-county. One of the farms had heavy infestation by root knot nematodes. The root samples were analysed at Egerton University Department of Biological Sciences laboratory. Perineal patterns cut on the nematodes indicated that the root knot nematode present was *Meloidogyne hapla*. The efficacy of sodom apple fruit extracts against the root knot nematodes was tested under greenhouse and field conditions. 300 grams of sodom apple fruits (*Solanum incanum*) were blended and dissolved in 1 liter of water. Four dilutions were obtained through serial dilution (100%, 50%, 25% and 12.5%). The extracts were applied on chilli plants that were pre-infected with *Meloidogyne* spp. at the rhizosphere. Evaluation with sodom apple fruit extracts after treatment of plants showed that there was significant effect on plant heights, number of galls, stem diameter and leaf number in chilli. Highest heights were recorded in the 50% treatment of the field experiment. The lowest number of galls in the field experiment was in the 25 % treatment. Evaluation after treatment of plants with different concentrations of *T. viride* isolates showed that there was no significant effect on plant heights, number of galls, stem diameter or leaf number in chilli. The nitrogen and phosphorous levels indicated there was significant difference in the nutrient levels when different treatments of *Trichoderma viride* and sodom apple fruits were applied on chilli with root knot nematodes. All treatment effects were determined by one way ANOVA using SAS program (Version 9.3). The characterization of the nematodes has provided information that will aid in proper control of nematodes affecting chilli leading to improved livelihoods. Since the sodom apple fruit extracts were found to be effective in managing the root knot nematodes, they can be recommended to farmers as part of the integrated management system of pests in chilli by formulation of nematicides.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
EPI	Egg Parasitic Index
EU	European Union
HCL	Hydrochloric acid
J2	Juvenile Larvae Stage 2
LDL	Low Density Lipoprotein
LSD	Least Significant Difference
MB	Methyl Bromide
MIC	Minimum Inhibitory Concentrations
nm	nanometers
Rpm	Revolutions per minute
SAS	Statistical Analysis System
USA	United States of America

CHAPTER ONE
INTRODUCTION

1.1 Background information

Plate 1: Potted, fruiting chilli plant
www.pinterest.com, Retrieved on 11th October 2017

Chilli (*Capsicum annum*) is a tropical and sub-tropical crop which has been under cultivation for many thousands of years. The chilli plant is unique due to its edible berries that have a characteristic pungent taste. This is attributed to the presence of an organic compound capsacain (Lee *et al.*, 2004). This compound produces heat sensations in most mammals including man. Chillis are widely distributed mainly due to their seed dispersal by birds. Birds are insensitive to capsacain and therefore feed comfortably on the chilli berries (Perry *et al.*, 2007). The seeds are indigestible and are therefore passed whole through feacal waste.

Chilli is used as a food additive in form of dried chilli powder, as a sauce, as a vegetable, as a medicine source and as a biological weapon (Wasbir, 2010).

India is the largest producer and consumer of chilli in the world. Chilli contributes to the economies of many countries and is the major income earner in Bhutan, South Asia (Biradar *et al.*, 2013).

One in acre in Kenya has a production potential of 10 tonnes. One tonne has a market value of Ksh 400,000 (Agro-Environment Initiative, 2011). The average production potential per acre in India is 8 tonnes. One tonne in India has a market value of Ksh 120,000 (Jagtap *et al.,* 2012). Production data varies in different countries as shown in Table 1.

Table 1: Chilli production data in selected countries

Country	Chilli Production in hg/ha, 2014
Algeria	36,449
Australia	188,769
Israel	616,116
Kenya	11,782
India	84,916
Jamaica	135,848
Mexico	190,474
Nigeria	77,607

www.fao.org/faostat/en/#data/QC, Retrieved on 11th October 2017

Capsicum annum, commonly referred to as pepper or chilli in many countries, is a common food additive world over. Chilli has several localized names that differ from region to region, *Pilipili* in Kenya, *Chipotle* in Mexico and *Solo* in Bhutan. Chilli contributes significantly to the economies of many countries. Chilli is considered the most important crop in Bhutan. Chillis have been used by many generations dating thousands of years ago. Archaeological discoveries proof that chilli was domesticated around 4000 BC. Such archaeological evidence has been collected in Ecuador (Perry *et al.,* 2007).

Jalapeno chilli is a chilli variety grown extensively in Mexico consumed by almost all the population (Hahn, 2002). The fruits are fried whole or ground and suspended as a hot sauce. These chilli fruits are added as flavours. However in some cultures chilli are eaten whole.

Chilli can be dried and preserved for a long period of time in powder form or as whole fruits. These products are consumed locally or exported from the areas of production. India remains the highest exporter of chilli and other vegetables (Parihar *et al.,* 2010).

Chilli leaves are used as a vegetable. The leaves are sour but not as pungent as the fruits. Besides causing pungency in dishes it also imparts a red colour to dishes. Chillis are a rich source of Vitamin A, C and E (Mishra *et al.*, 2009). Root knot nematodes (*Meloidogyne* spp.) cause high losses in many agricultural crops worldwide. Chilli is attacked by root knot nematodes which reduce the yields significantly.

The use of chemicals to control nematodes has been faced with challenges due to its effects on the environment. The withdrawal of methyl bromide as a soil fumigant has triggered research into alternative methods of controlling nematodes.

In the recent past, different plant extracts have been used in the control of various diseases and pests in crops. Various leaf extracts of noxious weeds such as *Solanum xanthocarpum* and *Argemone maxicana* have been used as bare-root dip treatment for the management of plant-parasitic nematodes (Javed *et al*., 2008).

In this study, the efficacy of sodom apple fruit extracts was tested on their ability to reduce root knot nematode infestation in chilli (*Capsicum annum*). Biological control methods in recent years have been adopted as more environmentally friendly techniques of nematode control (Hafeez, 2000). Different fungal isolates have been tested for antagonism against root knot nematodes. The genus, *Trichoderma* is a common filamentous fungi found in most soils (Behzda *et al*., 2008). The myco-parasitic abilities of different strains of *Trichoderma* against microorganisms such as nematodes have been reported (Al-Fattah, 2007). *Trichoderma harzianum* has been used to control *Meloidogyne javanica* (Baharullah *et al*., 2008). In this study, the efficacy of *Trichoderma viride* isolates was tested on its ability to reduce root knot nematode infestation in chilli (*Capsicum annum*).

1.2 Statement of the problem

Chilli cultivation has been greatly hampered by nematode infestation. Losses due to nematode infestation in chilli are about 23%. This was reported in Punjab, Pakistan (Safdar, 2012).

Farmers in Nakuru County are incognizant that their yields are reduced by nematode infestation. There is also lack of nematode resistant chilli varieties; this has reduced quality and yields of chilli resulting to devastating losses to farmers and the County Government of Nakuru.

1.3 Objectives

1.3.1 General objective

To improve the production of chilli using some nematode management practices in Nakuru County.

1.3.2 Specific objectives

1. To characterise root knot nematodes affecting chilli in Nakuru County.
2. To investigate nematicidal activities of sodom apple fruit extracts against root knot nematodes infesting chilli.
3. To investigate the effect of *Trichoderma viride* when used as a bio-agent against root knot disease in chilli.
4. To investigate the effect of root knot nematodes on the uptake of nitrogen and phosphorous when sodom apple fruit and *Trichoderma viride* extracts are applied in chilli.

1.4 Hypotheses

1. There is more than one *Meloidogyne* species attacking chilli in Nakuru County.
2. Sodom apple fruit extracts have no nematicidal effect against root knot nematodes infecting chilli.
3. *Trichoderma viride* has no nematicidal effect on the root knot nematodes infecting chilli.
4. Root knot nematodes have no effect on the uptake of nitrogen and phosphorous in chilli when sodom apple fruit and *Trichoderma viride* extracts are applied.

1.5 Justification

Chilli production has gone commercial in many areas of Kenya. Most soils have become infested with root knot nematodes which have been known to cause tremendous losses in many food crops including chilli. Whereas the losses caused by nematodes in Kenya have been documented for other vegetables, there is no available data for losses in chilli. These nematodes need to be controlled effectively. Nematicides have been used widely to control root knot nematodes. In recent years the need to formulate more environmentally friendly control methods has triggered research in bio-control and use of soil amendments as possible control methods of root knot nematodes. Various plant extracts and antagonistic organisms have been tested against root knot nematodes. The study focused on the development of a more sustainable, cost effective and environmentally friendly nematode management strategy. This will ultimately result in increased yields and reduced nematode management costs for chilli producers in Nakuru. The study generated data that will be helpful in formulating better management methods against root nematodes of chilli in Nakuru County. This will lead to improved yields which will result in improved livelihoods.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin of chilli

Chilli belongs to the genus *Capsicum* in the family *Solanaceae*. Chilli is thought to have originated in Mexico. There exist many wild varieties of *Capsicum*, however only five varieties are under cultivation. Of the five, *Capsicum annum* and *Capsicum frutescens* are the most cultivated. Most varieties under cultivation belong to the species *Capsicum annum* (Berke, 2002). These varieties include, Jalapeno, Long cayenne, Oiseau and Green hot.

2.2 Economic importance of chilli

Chilli cultivation in many countries has gone commercial. The great and widening demand for chilli and its products has led to increased cultivation of the crop from simple potted plants for home use to thousands of hectares for commercial use (Hussain, 2013).

2.2.1 Chilli as a vegetable

Communities in India eat chilli whole as a vegetable. The chilli fruit is used as a meal condiment where it is slightly fried whole on simmering oil. These fried chillis are then served with chicken or with other accompaniments such as rice or beef according to the recipe. Chilli leaves are also cooked and served as a sour vegetable. Chilli leaves are however not as sour as chilli fruits. This result to more use of chilli leaves as a vegetable compared to chilli fruits (Kumar *et al.*, 2006).

Its food additive qualities are expressed in diverse uses in recipes across the world. These flavours range from chopped fresh cut chilli, powder dried chilli and hot sauces. Chilli is added directly to food or is fried together with onions or exclusively on its own. Chilli sauce is a common household food additive that is used in practically all foods (Biradar, *et al*., 2013).

The secret of chilli use is in its refreshing capability. It is easily available and has a unique hot taste. In fact food tastes hotter with chilli flavour. This is due to its ability to activate heat sensors in the mouth (Bosland, 1996).

2.2.2 Chilli as a source of medicine

The alkaloid capsacain has high medicinal value. It dilates blood vessels therefore reduces the chances of high blood pressure.

Carotenoids found in chilli fruits (b-carotene, acyl derivatives of capsanthin, acyl derivatives of capsorubin) have been shown to be inhibitants of Low Density Lipo-proteins (LDL) oxidation in vitro with probable lowering of the "atherogenic" LDL sub-fraction production (Medvedeva *et al.,* 2003).

Capsacain has recently been tried as an intra-vesical drug for overactive bladder (bladder cancer) and it has also been shown to induce apoptotic cell death in many cancerous cells (Lee *et al.,* 2004). Chilli pungent characteristic is used in treatment of psychological conditions. High amounts of chilli are administered to patients. The severe pungent feeling acts as a therapeutic agent in their healing (Kim, 2012; Biradar *et al*., 2013).

Capsanthin and capsorubin (major carotenoids exclusively present in chilli fruits) can improve the cytotoxic action of anticancer chemotherapy. They are considered to be potential resistance modifiers in cancer chemotherapy (Maoka *et al.,* 2001).

2.2.3 Chilli as a biological weapon

The fact that capsacain is highly reactive with the skin leading to a momentous hot sensation has been utilized by various security arms including the military, police force and private security firms. During control of unruly crowds such as rioters or hooligans, chilli is mixed with hot water and stored in large water containers mounted on trucks. The chilli spray is directed at the unruly crowd using massive generators that produce a literal chilli rain through multiple horse pipes. The results are immediate with great agony of rioters who writhe in pain on the ground as they experience the momentous torture of capsacain on their skins (Wasbir, 2010; Bosland, 1996).

This method has been used widely by the China police and other police arms in the world to control huge unruly groups of people. Within minutes of torrents of chilli rain, the crowd is hampering for safety in all directions. This actually averts great damage that looms in such situations. Chilli sprays are also used as mild personal defense weapons. The Maya threw chilli powder into the eyes of young girls who stared at boys or men and they squirt fruit juice on the private parts of unchaste women (Bosland, 1996).

2.3 Chilli cultivation

Chilli requires a warm and humid climate for its best growth and dry weather during the maturation of fruits. Chilli crop grows well in tropical and sub-tropical regions, but it has a wide range of adaptability and can withstand heat and moderate cold to some extent.

The crop can be grown over a wide range of altitudes from sea level up to nearly 2100 meters above sea level. It is generally a cold weather crop, but can be grown throughout the year under irrigation. Black soils which retain moisture for long periods are suitable for rain fed crop whereas well drained chalka soils, deltaic soils and sandy loams are good under irrigated condition (Mohammud *et al.*, 2011).

Chilli cultivation requires tropical temperatures of about 28 ^{o}C. Chilli requires fertile loam soils with a good proportion of humus. Chilli requires slightly higher temperatures to flower. Green houses are therefore highly suitable in chilli cultivation. In the green house, the chilli requires heat regulators to maintain the temperature at around 28 ^{o}C (Sharif, 2003).

Chilli is easily cultivated in the field with simple management skills. Most of the chilli under cultivation is in tropical and sub-tropical countries. Chilli is a day crop. However farmers in temperate countries grow chilli in the field but devise techniques of raising the temperature for growth. Such chilli plants are grown in the backyards where they are conditioned with special bulbs that emit proper amounts of heat and light. Chilli has also been grown indoors in pots. The indoor temperature is slightly higher offering conducive environment where growth occurs normally (Mohammud *et al.*, 2011).

Low temperatures lead to lack of flowering in chilli. With the temperatures being optimal the chilli can produce fruits throughout the summer and even extend if temperature remains optimal. A chilli plant can produce fruit for many years but the yield depreciates with time. Chilli farmers can harvest four generations from one crop then plant another new crop (Baudoion, 2002).

2.4 Chilli diseases

Chillis are attacked by various diseases which reduce their production. These include:

2.4.1 Chilli anthracnose

Anthracnose disease caused by *Colletotrichum capsici* is one of the major economic constraints to chilli production worldwide, especially in tropical and subtropical regions. Infected fruits have small circular spots while the stems have dieback symptoms. Although the management and control of anthracnose disease are still being extensively researched, commercial cultivars of *Capsicum annum* that are resistant to the pathogens that cause chilli anthracnose have not yet been developed (Po po *et al.*, 2008).

2.4.2 Grey mold

Grey mold is the more common name for the fungal infection by *Botrytis cinerea*. Its infection is visible when the fruit starts to ripen, sugar content rises and the pods go to a brown mush. Such infections encourage opportunistic bacterial diseases. Bacterial leaf spot is caused by the seed borne bacterium *Xanthomonas campestris* pv. *vesicatoria.* It is one of the most serious bacterial diseases affecting chilli. This disease first appears as small water soaked areas that enlarge up to a quarter inch in diameter. The disease spots have black centers and yellow halos. The spots are depressed on the upper leaf surface, whereas on the lower surface the spots are raised and scab like (Mark and Julian, 2014).

Bacterial soft rot is caused by bacterium *Erwinia carotovora* pv. *carotovora* and affects chilli pods. The internal tissue softens before eventually turning into a watery mass with a foul smell. Bacterial Wilt is caused by the bacterium *Pseudomonas solanacearum*. The first symptoms start with the wilting of the leaves. After a few days, a permanent wilt of the entire plant results, with no leaf yellowing (Ushakiran *et al*., 2006).

2.4.3 Cercospora leaf spot (frog eye)

Cercospora leaf spot (Frog eye) is caused by the fungus *Cercospora capsici* and is worst under extended warm, wet conditions. This disease is characterised by small brown circular leaf lesions that have a watery appearance, excessive leaf drop may occur (www.pestnet.com, Retrieved on 11th October 2017).

2.4.4 Phytophthora blight (Chile wilt)

Phytophthora blight (Chile wilt) is caused by a water borne fungus *Phytophthora capsici* and is generally observed in wet waterlogged areas. The fungus can invade all plant parts causing at least three separate syndromes: leaf blight, fruit rot, and root rot.

Plants suffering from this condition often wilt and die, leaving brown stalks and leaves and small, poor-quality fruits (Sanogo, 2003).

2.4.5 Powdery mildew

Powdery mildew is caused by the fungus *Leveillula taurica* and primarily affects leaves on chilli plants during warm wet conditions. Diseased leaves eventually drop off, leaving pods susceptible to sunscald (Ranjale *et al*., 2012).

2.4.6 Verticillium wilt

Verticillium wilt is caused by soil borne fungus *Verticillium dahliae*, it is a soil borne fungi which can infect chilli plants at any growth stage. Plants may show a yellowing of leaves and stunted growth. As the disease progresses, the plants can shed leaves and may finally die (Sanogo, 2013).

2.4.7 White mold

White mold is caused by the fungus *Sclerotinia sclerotiorum*. It causes blighting or rotting of any above or below ground plant parts. At first, the affected area of the plant has a dark green, greasy, or water-soaked appearance. On stems, the lesion may be brown to grey in colour. If the humidity is high, a white, fluffy mold growth may appear. Symptoms include stunted plants, distorted fruit, and yield reduction (Mark and Julian, 2014).

2.4.8 Damping-off disease

Damping-off is caused by poor seed quality, improper planting depth, high salt concentrations, wet seed beds or severe nutrient deficiencies. Several fungi such as *Pythium*, *Rhizoctonia* and *Fusarium* are associated with this infection. Seedlings fail to emerge (pre-emergence damping-off), small seedlings collapse (post-emergence damping-off), or seedlings are stunted (www.worldofchillies.com, Retrieved on 11th October 2017).

2.4.9 Tobacco etch virus (TEV)

Tobacco etch virus (TEV) is caused when infected aphids and other insects come into direct contact with the plant. Symptoms include dark green vein bands, leaf distortion and stunted growth. Tabasco chilli plants are particularly susceptible to this disease and often wilt and die (Sushel *et al.,* 2010).

2.4.10 Tobacco mosaic virus (TMV)

Tobacco mosaic Virus (TMV) is a highly infectious and persistent disease. It is carried by tobacco in cigarettes and is spread mechanically, by infected hands touching tools or plants. Symptoms can include curling leaves, spotted or mottled fruit, stunted plants and excessive leaf drop (Nurhayati, 2014).

2.4.11 Root knot nematodes

Root knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most destructive pests of agricultural crops. They are worldwide in distribution having a very wide host range.

Average crop losses due to these nematodes in the tropical and sub-tropical countries are 15% annually. However, in vegetable crops, these losses may reach up to 50-80% (Oka *et al*., 2000).

The root-knot nematodes (*Meloidogyne* spp.) are important pathogens of several solanaceous crops, especially chilli, potato, and tomato (Baharullah *et al.*, 2008). Roots attacked by this parasite exhibit characteristic root galls, and infected plants grow poorly or even die because of vascular dysfunction. As sedentary endoparasites, *Meloidogyne* spp. have complex trophic relationships with their host plant and induce specialized feeding structures known as giant cells, which are essential to the nutrition and development of the nematode (Hussey and Janssen, 2002).

The species are characterised on the basis of their perineal patterns, the morphology which is located at the posterior body region of adult females (De Ley and Blaxter, 2002). The posterior region comprises the vulva, anus, lateral lines, phasmids, tail and surrounding cuticular striae (De Ley and Blaxter, 2004), which differ in *Meloidogyne* spp. and are useful for identification as summarized in Table 2.

Table 2: Taxonomic characters of perineal patterns of the four common root knot nematodes

Species	Dorsal arch	Lateral field	Striae	Tail terminus
M. incognita (Koffoid and White)	high, squarish	lateral ridges absent, marked by breaks and forks in striae	coarse, smooth to wavy, sometimes zigzaggy	often with distinct whorls
M. javanica (Treub)	low, rounded	distinct lateral ridges	coarse to slightly wavy	often with distinct whorls
M. arenaria (Neal)	low, rounded, indented near lateral fields	lateral ridges absent, marked by short, irregular and forked striae	coarse to slightly wavy	usually without distinct whorls
M. hapla (Chitwood)	low, rounded	lateral ridges absent	fine, smooth to slightly wavy	whorls absent , marked by subcuticular punctations

Eisenback, 1985.

Symptoms due to the root knot nematode infestation are manifested in above and below ground parts of the plants. Above ground symptoms include yellowing, stunting and wilting. Underground symptoms are more typical and include galls, rotting, necrosis, cracking, distortion and bushy roots (Barker *et al.*, 1985).

2.5 Management of root knot nematodes in chilli

2.5.1 Use of chemicals to control root knot nematodes

Root knot disease management is generally achieved through the use of nematicides or use of resistant crop varieties. A wide range of nematicides are available. Large scale use of nematicides in nematode management has declined worldwide.

This is due to the toxic effect of nematicides to humans and the entire ecosystem. In addition, they are relatively unaffordable to many resource poor small-scale farmers because of the high cost (Chitwood, 2002). Until 2005, methyl bromide (MB) was used to disinfect soils to control nematodes and to reduce the effects of fatigue caused by repeated monocultures. Since that time, MB has been replaced by a mixture of 1, 3-dichloropropene and chloropicrin, but these, too, will shortly be banned by EU legislation (Martínez *et al.*, 2011).

Several problems are associated with the use of chemicals such as their poor penetration into the nematode eggs, rapid leaching and degradation, high cost and above all, chemicals are a serious threat to the environment. Soil fumigation with methyl bromide has been widely practiced in the USA for pre-plant control of nematodes (Taylor, 1994). Common commercial nematicides include Metam-sodium, Dazomet, Thionazin, Aldicarb, Oxamyl among others (www.fao.org, Retrieved on 11th October 2017).

2.5.2 Use of solarisation to control root knot nematodes

Soil solarisation for nematode control has been applied in a number of crops and locations characterized by hot, clear weather. Many studies have proved that solarisation greatly reduces nematode populations. Soil subjected to solarisation shows reduced nematode population. Production in chilli grown with solarised soil is enhanced (Guerrero *et al.*, 2013). Temperatures that vary between 22 ^{O}C and 42 ^{O}C considerably reduce *M. incognita* J2 populations and eggs (Wang and McSorley, 2008).

2.5.3 Biological control of root knot nematodes

A large number of bio-control agents have been tested to manage root-knot nematodes with encouraging results (Sharon *et al*., 2001). *Trichoderma* species (*Trichoderma atroviridae*, *Trichoderma harzianum*, *Trichoderma rossicum*, *Trichoderma tomentosum*, *Trichoderma virens* have been used to control plant-parasitic nematodes. These products have been commercialized (Hafeez *et al.*, 2000).

2.5.4 Use of selected botanicals for control of root knot nematodes

The nematicidal effect of several plants has been well reported by various researchers. Various leaf extracts of noxious weeds such as *Solanum xanthocarpum* and *Argemone maxicana* have been used as bare-root dip treatment for the management of plant-parasitic nematodes. Neem based nematicides are already in the market (Javed *et al*., 2008).

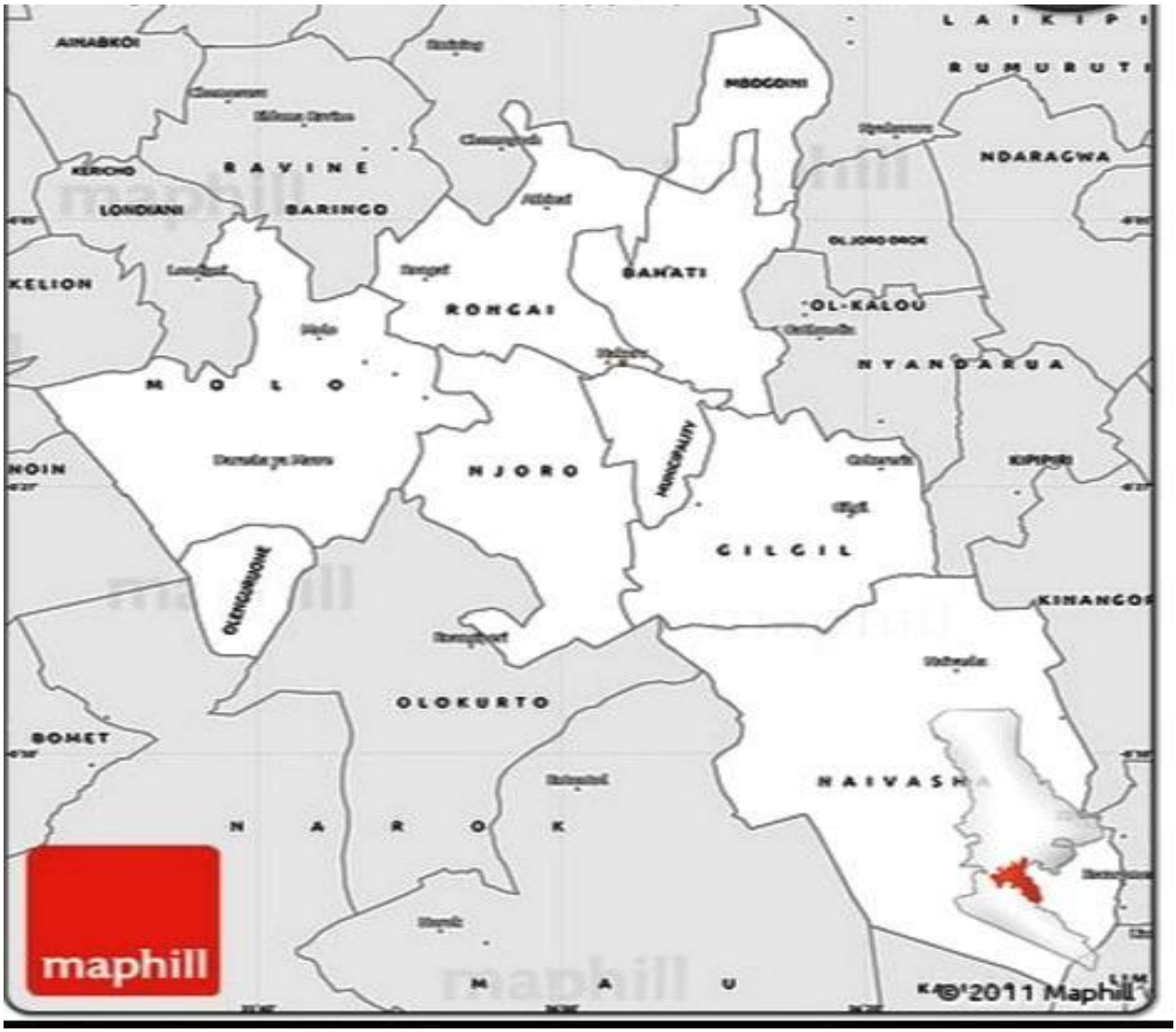
2.5.5 Use of resistant varieties

The use of resistant varieties is the most economical and environmentally friendly method of controlling nematodes effectively (Tenson *et al*., 1999). The chilli variety *Pusa Jwala* was reported to be moderately resistant compared to *Pusa Sadhabahar, PC-1, Mathania local* and *Jaipur local* (Abhiniti *et al*., 2012).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of study area

Nakuru County is one of the counties in Kenya. Nakuru is the fourth largest county in Kenya after Nairobi. It is situated at an altitude of 1871m above the sea level. The average rainfall is 895 mm with average temperatures of 17.5 ^{o} C. (www.en.climate-data.org.com, Retrieved on 10th October 2017)



NAKURU COUNTY MAP- White highlighted area- Study Site

Map source: Maphill

3.2 Survey and sampling

A survey was conducted in seven sub-counties of Nakuru County. The sub counties included Njoro, Molo, Bahati, Subukia, Rongai, Naivasha and Gilgil. At least three farms were sampled per sub-county. The infected plants were uprooted and samples were put in labeled polythene bags for nematode extraction and identification. Samples were preserved in a refrigerator at 5 ^{O}C for subsequent analysis.

3.3 Characterisation of nematodes

Characterisation of nematodes was done by cutting perineal patterns. Adult females were teased out of clean roots using dissecting needles and put in a petri-dish with a little amount of water. Adult females have distinct patterns in their posterior region which are absent in males. The posterior side was cut off with a surgical blade and trimmed so that the perineal area remained in a piece of cuticle only 5-10 times its area. The inner tissues were removed carefully. The perineal patterns were transferred temporarily to a drop of lactophenol cotton blue 0.03% on a cavity glass slide. 5-10 perineal patterns were done per sample. These perineal patterns were then transferred to a permanent mount in which lactophenol cotton blue 0.03% was used. The slides were labeled after putting cover slips**.** Observations were done under different magnifications using a good stereoscopic microscope with a range of magnifications (X10, X40 and X100) and a fairly flat field and a good resolution. This method is as described by Taylor and Netscher (1974). Nematodes were correctly classified using the guide to the four most common species of *Meloidogyne* spp. with a pictorial key as prepared by Eisenback *et al.* (1981).

3.4 Nematode multiplication

Screening of chilli varieties against root knot nematodes was done. Oiseau variety was the most susceptible compared to Jalapeno, Cayenne and Green hot varieties. Chilli seedlings (Oiseau variety from Technisem, France) were raised in a greenhouse for a period of one month. The seedlings were then transferred to pots in a greenhouse. Nematodes were collected from chilli plants in Nakuru County. Nematode population was multiplied in the chilli over a period of 4 months. This nematode population was used as the stock in the experiment. Heavily galled roots from infected chilli were mixed with the soil around the rhizosphere of the seedlings. Regular watering was done to give a conducive environment for nematode infestation.

Galls were extracted from infected roots after three months and were used for further increase of the nematode stock as described by Hussey and Barker (1973).

3.5 Preparation of extracts

a) Preparation of sodom apple fruit extracts

The ripe and unripe fruits of sodom apple were washed with distilled water and blended with distilled water. This was to ensure maximum phytochemicals were collected. Three hundred grams of fruits were dissolved in one liter of water. This was the optimum ratio to get a semi fluid extract which was not too viscous. The mixture was blended to get the crude juice. The extracted juice was passed through a double layered cheese cloth. This is a modified procedure as described by Ushakiran (2006).This formed the standard 100% plant extract. Four dilutions were derived by serial dilution as described by Pavaraj *et al*., (2012).

b) Preparation of *Trichoderma viride* extract

Fungal colonies of a stored pure culture of *Trichoderma viride* from the Egerton University Department of Biological Sciences were multiplied on potato dextrose agar plates at 25 ^{o}C over a period of one week. Sub-culturing was done to multiply the fungus. The stock solution of *Trichoderma viride* was derived by scrapping fungal colonies of pure cultures from three plates and suspending them in 200 ml of water. The solution was put in an orbital shaker set at 100 rpm for fifteen minutes. The concentration of the stock suspension was determined using a hemocytometer. It was found to be 2.0 x 10^{6} spores per millimeter cubed and was labeled 100%. The consequent treatments were diluted to 50 %, 25% and 12.5% (Pavaraj *et al.*, 2012).

3.6 Experimental design and application of inoculum

The experiment was laid in completely randomized block design in the field and complete randomized design for the green house with three replicates in each experiment, each replicate had six pots.

Soil that was used in the experiment was steam sterilized at 100 ^{o}C in a metal drum. The soil was allowed to cool and was put in 5000 cm ^{3} plastic pots. The soil in each pot was treated with nematodes eggs suspended in 50ml of water. There were an approximate number of 5000 nematode eggs/ ml.

3.7 Application of treatments

In the first experiment, six treatments of sodom apple fruit extract were imposed on the soil bearing nematodes in each replicate. These treatments were applied the same day when nematodes were applied. The treatments were applied once.

The four levels of sodom apple fruit extracts derived earlier were used (100% labeled S1, 50% labeled S2, 25% labeled S3, and 12.5% labeled S4). The treatments were in volumes of 50ml.

The positive control was a nematicide by the market name Nimbecidine (Osho Chemicals, Kenya) labeled S6. The active ingredient is azadiractin (0.03%) and neem oil (90.57%) applied at the rate of 6-8ml per meter squared. The negative control used was tap water labeled S5. Four seedlings were planted per pot one week after application of treatments.

In the second experiment, six treatments of *Trichoderma viride* were used. These treatments were applied the same day when nematodes were applied. Four levels of *Trichoderma viride* derived earlier were used (100% labeled T1, 50% labeled T2, 25% labeled T3, and 12.5% labeled T4). The treatments were in volumes of 25 ml. The positive control was a nematicide by the market name nimbecidine labeled T6. The active ingredient is azadiractin (0.03%) and neem oil (90.57%) applied at the rate of 6-8ml per meter squared. The negative control used was tap water labeled T5.

The treatments were applied once. Four seedlings of Oiseau variety were planted per pot one week after application of treatments. Watering was done twice per week. The greenhouse was not heated.

3.8 Measurement of parameters for the sodom apple and *Trichoderma viride* experiments

Data collection for heights, leaf numbers and stem diameter was done monthly after planting of seedlings and the data was recorded thrice. The formed root galls were collected after two months after planting to allow gall formation. The galls formed on the roots of each plant were counted. One plant was uprooted per pot. The data was recorded twice.

3.9 Nitrogen and Phosphorous level determination

Chilli leaves for nutrient analysis were harvested after two months. The leaves were oven dried at 60 ^{o}C for 12 hours. They were then crushed using an electric grinder and suspended in water. Total nutrient analysis was done using Kjeldahl oxidation method as used by Okalebo and Woomer (1993).

The determination of nitrogen level was done by putting 5 mls of an aliquot to a Kjeltec auto distillation apparatus (Model 2200). The ammonia in the distillate was collected in a receiver with excess boric acid and a methyl red indicator. Blank determination was done by digesting reagents in place of the sample and distilling and titrating with N/70 HCl as for the samples. The percentage of Nitrogen (N) in the plant tissue was determined by the following formula;

$$\%N \text{ in plant sample} = \frac{\text{Corrected ml of N/70 HCl} * 0.2}{\text{Weight of sample in grams}}$$

(Okalebo and Woomer, 1993)

The determination of phosphorous level was done by putting 5 mls of supernatant wet-ashed digested solution to a 50 ml volumetric flask using a pipette. Twenty ml of distilled water was added to each flask. Ten ml of ascorbic acid was added, then made to 50 ml with distilled water.

The solution was allowed to stand for 1 hour to permit full colour development. The standard and sample absorbance (blue colour) was measured at 880 nm wavelength using a spectrophotometer (Model Pharmacia Biotech).

The total phosphorous percentage was computed as follows;

$$\% \text{ P in plant sample} = \frac{C * 0.05}{W}$$

Where C is the corrected concentration, * means multiplication, P is phosphorous and W is the weight of the sample in grams.

(Okalebo and Woomer, 1993)

3.10 Statistical analysis

Treatment effects were determined by one way ANOVA using SAS program (Version 9.3). Means and standard errors of the means were calculated for all data. Comparisons of means was done using LSD at a $p = 0.05$.

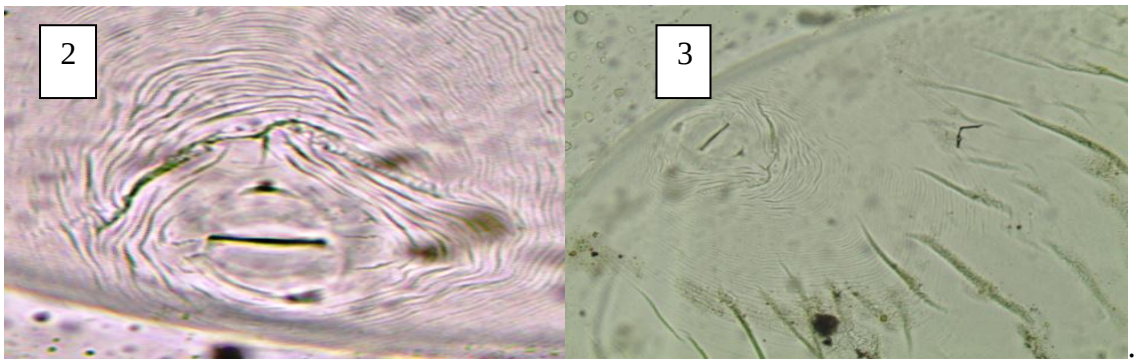
CHAPTER FOUR
RESULTS

4.1 Sampling and survey

The survey conducted encompassed the following sub counties of Nakuru; Njoro, Molo, Bahati, Subukia, Rongai, Naivasha and Gilgil. At least three farms were chosen randomly per sub-county. The farms in Naivasha, Bahati, Gilgil sub-county had cayenne varieties. The chilli did not have nematode infestation. In Subukia, farmers had planted Cayenne and Oiseau varieties; the chillis were not infested by nematodes. Farms in Njoro sub-county had Oiseau, Cayenne and improved Cayenne varieties. One of the farms with Oiseau variety had heavy infestation by root knot nematodes. Molo County did not have chilli farms. It was observed that many farmers in Nakuru County had difficulties in accessing good market for their chilli. There was over exploitation by middlemen which lowered the morale of farmers. This resulted to many farmers abandoning chilli farming.

4.2 Nematode characterisation

Root knot nematodes collected from this study are depicted in Plate 2-6. The perineal patterns were found to have an overall rounded hexagonal to flattened ovoidal shape; they had very fine striae and subcuticular punctuations in the smooth tail terminal area. The dorsal arch was found to be low and rounded. Lateral ridges were absent but the lateral fields were marked by irregularities in the striae. The dorsal and ventral striae often meet at an angle, and the striae are smooth to slightly wavy. Some patterns formed wings on one or both of the lateral sides. The perineal patterns had taxonomic features characteristic of *Meloidogyne hapla*.



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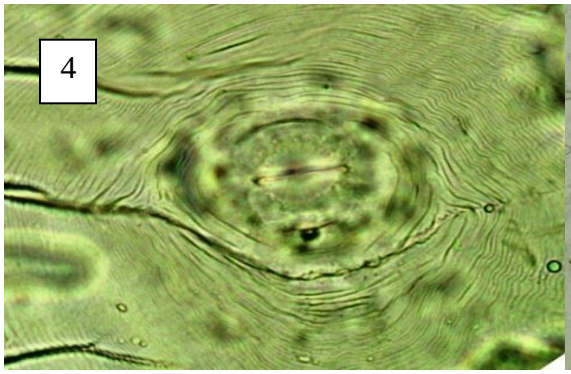

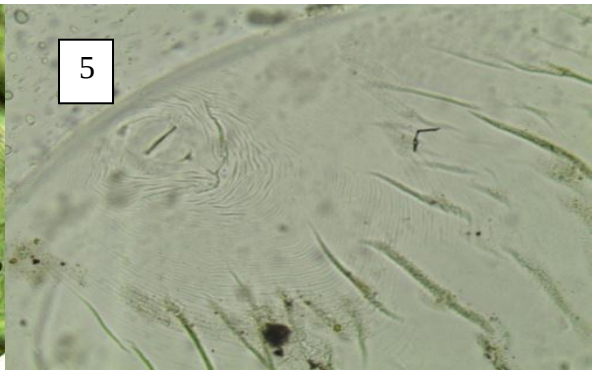

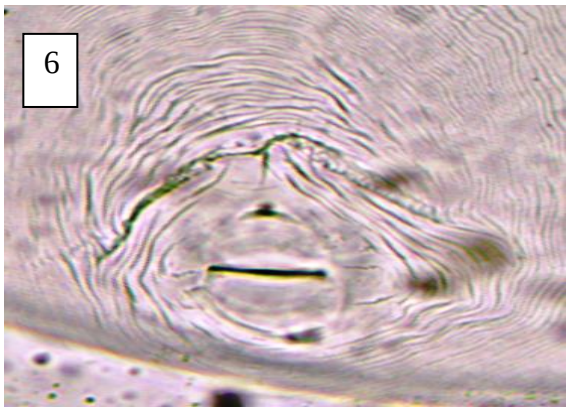

Magnification= 40x

Plate 2, 3, 4, 5, 6 - Perineal patterns (cross sections) of root knot nematodes collected in Nakuru County

All the plates depict different photographs of the patterns of *Meloidogyne hapla.*

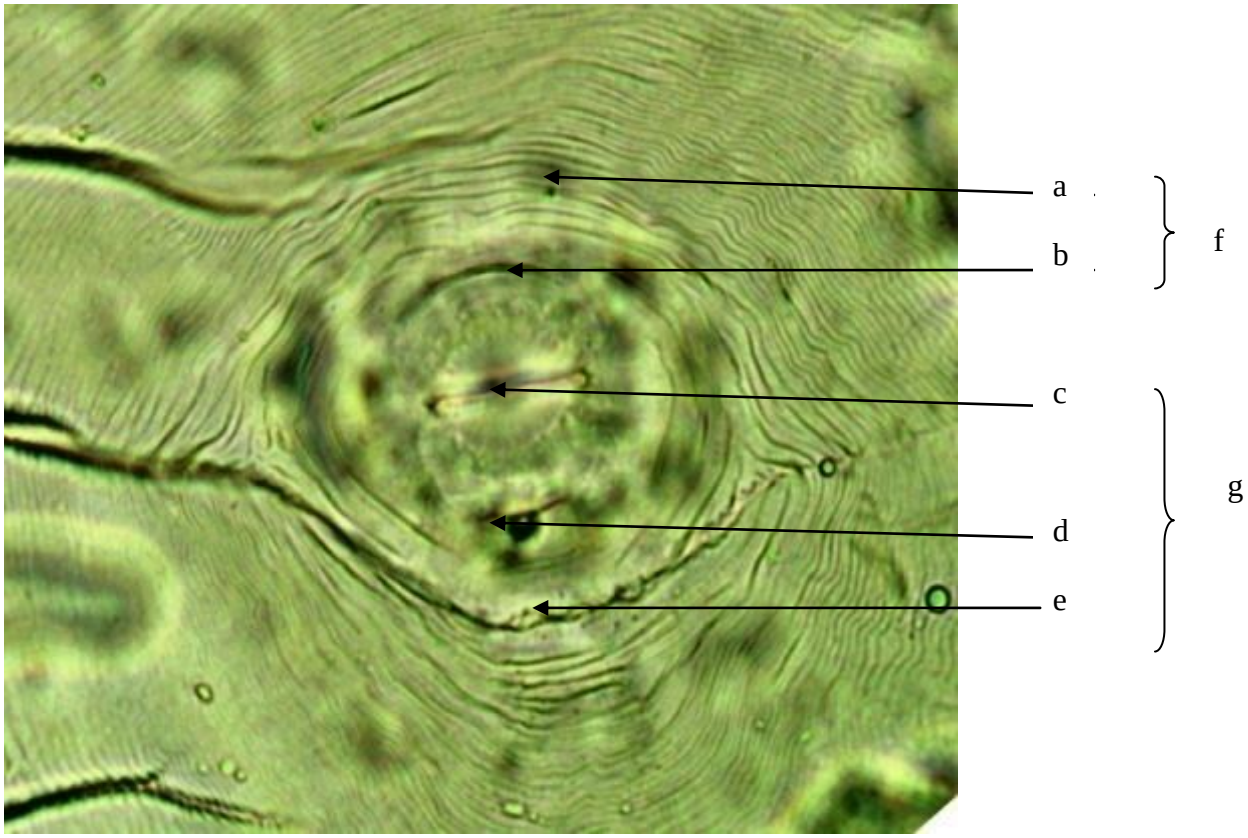

Plate 7: Features of perineal patterns

LEGEND

a-arch

b-tail tip

c-lateral line

d-anus

e-vulva

f-dorsal side

g-ventral side

Chilli that were infected with root knot nematodes had symptoms of deformed leaves, yellowing, stunting, wilting and general plant weakness (Plate 8).

Plate 8: Nematode infested chilli plants in the greenhouse

Chilli plants that were not infected by root knot nematodes were generally more vigorous than the infected ones. The leaves were also in normal shape and were more green (Plate 9).

Plate 9: Healthy chilli plants in the field

Healthy chilli roots did not have galls and were more branching and healthy (Plate 10).

Plate 10: Healthy chilli roots

Roots of chilli that had root knot nematodes had galls, rotting, necrosis, cracking and distortion (Plate 11).

Arrows point to galls on the chilli roots.

Plate 11: Galled roots of chilli that were infested by root knot nematodes

4.3 Sodom apple fruit extract experiment

a) Sodom apple fruit extracts greenhouse experiment

Significant difference in the mean heights was only observed during the first reading. The highest heights were recorded in the positive control (T6) followed by the 50% treatment (T2) and negative control treatment (T5) respectively. The lowest height reading was recorded in the 25% treatment (T3). During the second and third reading there was no significant difference in the mean heights (Table 3).

Table 3: Chilli mean heights in the greenhouse experiment

Treatments	Height 1	Height 2	Height 3
T1	9. 3b	19a	25a
T2	11ab	17a	20a
T3	8. 3b	14. 7a	25. 3a
T4	9. 7ab	12. 7a	11.3a
T5	10.3ab	17a	25. 3a
T6	12. 7a	21. 7a	24a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

There was no significant difference in the mean number of leaves, stem diameters and number of galls when different treatments of sodom apple fruit extracts were used in the greenhouse experiment.

b) Sodom apple fruit extracts field experiment

There was significant difference in the mean heights when different treatments of sodom apple fruits extracts were used. During the first reading, the 50% treatment (T2) and the 100% treatment (T1) had the highest mean heights. The lowest mean height was in the 25% treatment (T3). During the second reading, the highest mean height was in the 50% treatment (T2) while the lowest mean height was in the 12.5% treatment (T4). There was a general increase in height in comparison with the previous reading. During the third reading, the 100% treatment (T1) had the highest mean height while the 25% treatment (T3) had the lowest mean height. The 25% treatment (T3) had the lowest mean height (Table 4).

Table 4: Chilli mean heights in the field experiment

Treatments	Height 1	Height 2	Height 3
T1	15a	20.3ab	25.7a
T2	15a	21.7a	23.3ab
T3	10.7b	16.7b	18.7b
T4	13ab	16.7b	20.7b
T5	13ab	20.3ab	22.7ab
T6	11.7ab	18.3ab	23ab

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

There was significant difference in the mean number of galls recorded during the first reading. The highest number of galls was recorded in the 100% treatment (T1) followed by the 50% treatment (T2). The lowest number of galls was recorded in the 25% treatment (T3) and the negative control (T5). During the second reading, the highest number of galls was recorded in the positive control (T6) followed by the 12.5% treatment (T4). The lowest number of galls recorded was in the 25% treatment (T3) followed by the 100% treatment (T1) (Table 5).

Table 5: Means of chilli gall number in the field experiment

Treatments	Gall 1	Gall 2
T1	9.3a	10.7b
T2	5.3ab	11.3b
T3	3b	9b
T4	3.7b	15.3ab
T5	3b	11.3b
T6	4b	23.3a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

There was significant difference on the mean number of leaves when different treatments of sodom apple fruit extracts were used during the first reading only. There was a general increase in the mean number of leaves across the three readings (Table 6).

Table 6: Means of chilli leaf number in the field experiment

Treatments	Leaf 1	Leaf 2	Leaf 3
T1	20.7a	24.3a	34a
T2	24a	29.7a	27.7a
T3	15.7b	20.3a	29.3a
T4	21a	29.3a	38.3a
T5	16.3a	25a	52a
T6	16.3ab	33.7a	49.7a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

There was no significant difference in the means of stem diameters when different treatments of sodom apple fruit extracts were used in the field experiment.

4.4 *Trichoderma viride* extracts experiment

a) *Trichoderma viride* extracts greenhouse experiment

There was a steady increase in mean heights for all the chilli plants over the three months.

The positive control treatment had the highest mean height during the first reading while the negative control had the least height. During the second reading the 100% treatment had the highest mean height while the negative control had the least height.

During the third reading the positive control had the highest mean height while the 12.5 % treatment had the lowest mean height. However, there was no significant difference in mean heights recorded when different treatments of *T.viride* were used (Table 7).

Table 7: Chilli mean heights in the greenhouse experiment

Treatments	Height 1	Height 2	Height 3
T1	11. 3a	19. 7a	16.7a
T2	11. 7a	15. 3a	18.7a
T3	9. 7a	15.3a	13.3a
T4	9. 3a	15.3a	12.7a
T5	8a	14. 7a	13a
T6	12. 3a	18.3a	22.7a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

During the first reading the positive control had the highest mean leaf numbers while the negative control had the lowest mean leaf number.

During the second reading, the positive control treatment had the highest mean leaf numbers while the 100% treatment had the lowest mean leaf numbers. During the third reading the 100% treatment had the highest mean leaf numbers while the negative control treatment had the lowest mean leaf numbers. However, there was no significant difference in number of leaves recorded when different treatments of *T.viride* were used (Table 8).

Table 8: Means of chilli leaf number in the greenhouse experiment

Treatments	Leaf 1	Leaf2	Leaf3
T1	13. 3a	15. 7a	20 a
T2	14 a	20. 3a	19 a
T3	11 a	18. 7a	16 a
T4	10. 3a	20. 7a	14. 7a
T5	9. 7 a	18. 3a	13 a
T6	16. 3a	22. 7a	15. 3a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

During the first reading, the 50%, 12.5%, and positive control had the highest mean stem diameters. During the second reading, the 25% and negative control treatments had the highest stem diameters. The positive control had the least mean stem diameter during the second reading. During the third reading, the 50%, negative control and the positive control had the highest mean stem diameter.

However, there was no significant difference in stem diameter recorded when different treatments of *Trichoderma viride* were used (Table 9).

Table 9: Means of stem diameter in the greenhouse experiment

Treatment	Stem 1	Stem 2	Stem 3
T1	1. 7a	2. 3a	2. 3a
T2	2 a	2. 3a	2. 7a
T3	1 7a	2. 7a	2. 3a
T4	2 a	2. 3a	2. 3a
T5	1. 7a	2. 7a	2. 7a
T6	2 a	2 a	2. 7a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

During the first recording the highest mean number of galls was recorded in the negative control treatment while the least mean number of galls was recorded in the 100% treatment.

The second and third least mean number of galls was recorded in the positive control and 12.5% treatment respectively. During the second reading, the highest mean number of galls was recorded in the negative control treatment while the least mean number of galls was recorded in the 50% treatment. The second and third least mean number of galls was recorded in 12.5% and 25% treatment respectively. However, there was no significant difference in number of galls recorded when different treatments of *T. viride* were used (Table 10).

Table 10: Means of gall number in the greenhouse experiment

Treatments	Gall 1	Gall2
T1	4. 7 a	8 a
T2	7 a	5. 3 a
T3	7. 3 a	6. 7 a
T4	5. 7 a	6 a
T5	8. 7 a	16 a
T6	5.3 a	8. 7 a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

b) *Trichoderma viride* extracts field experiment

There was a steady increase in mean heights for all the chilli plants across the three months when heights were recorded. During the first recording, the negative control had the highest mean height; the positive control had the least height. The positive control treatment recorded the highest height at the end of the experiment. There was however no significant difference in mean heights recorded when different treatments of *T. viride* were used (Table 11).

Table 11: Chilli mean heights in the field experiment

Treatments	Height 1	Height 2	Height 3
T1	12a	18.7a	23.7a
T2	12a	16a	22.3a
T3	12.7a	17.7a	22a
T4	11.3a	18a	23.7a
T5	13.3a	18.7a	23a
T6	10.7a	17a	31.7a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

There was a steady increase in the number of leaves across the three data collection periods. The 25% *T. viride* treatment had the highest mean number of leaves for the first two months. By the end of the experiment the positive control treatment had the highest mean number of leaves followed by the 25% and 50% treatment respectively. However there was no significant difference in number of leaves recorded when different treatments of *T. viride* were used (Table 12).

Table 12: Means of chilli leaf number in the field experiment

Treatments	Leaf 1	Leaf 2	Leaf 3
T1	19.3 a	26 a	32. 7 a
T2	16. 3 a	20 a	35. 3 a
T3	25 a	29 a	37 a
T4	18. 7 a	25. 7 a	27. 7 a
T5	16. 7 a	24. 3 a	32. 7 a
T6	17 a	25. 3 a	52 a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

The diameter of stems was equal in the first recording except for the negative and positive control treatments which had the largest and uniform diameter. The diameters were uniform in the second reading.

The positive control and 25% treatment had the largest stem diameters at the end of the experiment. However, there was no significant difference in diameter of stems recorded when different treatments of *T.viride* were used (Table 13).

Table 13: Means of stem diameters in the field experiment

Treatments	Diam 1	Diam 2	Diam 3
T1	2a	2. 7a	3. 7a
T2	2a	2. 7a	3. 3a
T3	2a	2. 7a	4a
T4	2a	2. 7a	3. 3a
T5	2. 3a	2. 7a	3. 3a
T6	2. 3a	2. 7a	4a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

During the first reading the highest mean number of galls was recorded in the 100%, 25% and 12.5% treatment. The gall number increased sharply after the first reading. In the second reading, the highest numbers of galls were recorded in the positive control treatment; the 25% treatment was the second highest while the 50% treatment had the least number of galls. However, there was no significant difference in the number of galls recorded when different treatments of *T.viride* were used (Table 14).

Table 14: Means of gall numbers in the field experiment

Treatments	Gall 1	Gall2
T1	5. 7a	22. 3 a
T2	4 a	18. 7 a
T3	5. 7 a	41.7 a
T4	5. 7 a	21 a
T5	4. 3 a	21 a
T6	2. 3 a	54. 7 a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

4.5 Nitrogen and phosphorous determination

a) Sodom apple fruit extracts greenhouse experiment

There was significant difference in the nitrogen levels during the first reading. During the first reading, the highest nitrogen levels were recorded in the 12.5% treatment followed by the 25% and 100% treatment.

The lowest nitrogen level was recorded in the positive control treatment followed by the 50% treatment. There was no significant difference in the phosphorous levels (Table 15).

Table 15: Means of nitrogen and phosphorous levels in the greenhouse experiment

Treatments	N 1	N 2	P1	P2
T1	1.2ab	1.1a	782.2a	1335.6a
T2	0.9bc	1.2a	863.3a	1276.7a
T3	1.2ab	1.2a	803.9a	815.6a
T4	1.3a	1.1a	256.1a	848.3a
T5	0.9bc	1.1a	801.7a	732.8a
T6	0.8c	1a	1240a	256.7a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

Units of N= Percentage (%), P=ppm

b) Sodom apple fruit extract field experiment

There was significant difference in the nitrogen levels for the treatments during the first reading.

The highest nitrogen levels were recorded in the 25% treatment followed by the 100% treatment. The lowest nitrogen level was recorded in the 12.5% treatment followed by the 50% treatment (Table 16).

Table 16: Means of nitrogen and phosphorous levels in the field experiment

Treatments	N 1	N 2	P1	P2
T1	2ab	2.9a	3936a	1366a
T2	1.1b	1.2a	299a	2108a
T3	3.8a	2.5a	750a	2705a
T4	1.1b	1.2a	287a	1807a
T5	2ab	1.6a	2128a	4022a
T6	1.8b	1.6a	543a	1586a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

Units of N= Percentage (%), P=ppm

c) *Trichoderma viride* greenhouse experiment

There was no significant difference in the nitrogen and phosphorous levels when different treatments of *T. viride* were used in the greenhouse experiment (Table 17).

Table 17: Means of nitrogen and phosphorous levels in the greenhouse experiment

Treatments	N 1	N 2	P1	P2
T1	0.9a	1.2a	758.9a	849.4a
T2	1a	1.1a	297.5a	253.9a
T3	1.1a	1.1a	252.8a	1321.1a
T4	1.2a	1.1a	1320.6a	796.1a
T5	1.a	1.1a	921.7a	795a
T6	1.2a	1.1a	1296.1a	825a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

Units of N= Percentage (%), P=ppm

d) *Trichoderma viride* field experiment

There was significant difference in the nitrogen and phosphorous levels in the treatments. The nitrogen levels increased across the two months of data collection in the 100% treatment, and the positive control. In all the others the nitrogen level reduced in the second month.

During the first reading, the highest levels of nitrogen were recorded in the negative control treatment followed by the 12.5% treatment. The lowest nitrogen level was recorded in the 25% treatment followed by the positive control treatment. During the second reading the highest nitrogen level was recorded in the negative control treatment followed by the 100% treatment. The lowest nitrogen level was in the 25% treatment followed by the positive control treatment. During the first reading the phosphorous levels were highest in the negative control treatment followed by the 100% treatment. The lowest phosphorous level was recorded in the 50% treatment followed by the 25% treatment. During the second reading, the highest phosphorous levels were recorded in the negative control treatment followed by the 25% treatment. The lowest phosphorous level was recorded in the 100% treatment followed by the positive control treatment (Table 18).

Table 18: Means of nitrogen and phosphorous levels in the field experiment

Treatments	N 1	N 2	P1	P2
T1	1.6ab	1.7a	486.7b	478a
T2	1.2ab	1.2a	151.1b	1527a
T3	0.9b	0.9a	263.3b	2034a
T4	1.9ab	1.5a	414.4b	1682a
T5	2. 7a	2.2a	1255a	3535a
T6	0.9b	1.3a	281.1b	852a

a in the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least Significant Difference (LSD) test.

Units of N= Percentage (%), P=ppm

CHAPTER FIVE
DISCUSSION

5.1 Survey and sampling

It was noted that the farmers in Bahati sub-county applied cultural methods of control. These methods included ash and manure application. Farm yard manure is known to be antagonistic to root knot nematodes (Clark, 2007). The use of commercial nematicides and cultural methods in Gilgil used by the farmers was effective in controlling root knot nematodes. Nematicides such as Adicarb, Dazomet, Metasodium, Oxamyl and 1, 3 dichloropropene are effective against root knot nematodes (Muthamia, 2004). The use of many insecticides which are also lethal to nematodes would have resulted to death of root knot nematodes due to percolation of the insecticides in the soil. The Cayenne variety planted by most farmers could be resistant to root knot nematodes. Oiseau variety was probably susceptible due to its genetic make up. Resistance against *Meloidogyne* spp. has been reported in many food crops but it is not often used (Wesemael and Moens, 2009). The absence of chilli in Molo County was attributed to the very cold environmental conditions which did not support chilli farming (Sharif, 2003).

5.2 Characterisation and identification of *Meloidogyne* spp. attacking chilli in Nakuru County

The perineal patterns were found to have an overall rounded hexagonal to flattened ovoidal shape; they had very fine striae and subcuticular punctuations in the smooth tail terminal area unlike in *Meloidogyne incognita* which has high, squarish dorsal arch that often contains a distinct whorl. This is also not characteristic of *Meloidogyne javanica* which has lateral ridges that divide the dorsal ventral striae. These perineal patterns also did not fit the description for *Meloidogyne arenaria* perineal patterns which are distinguished by a low dorsal arch that is slightly indented near the lateral fields to form rounded shoulders. The perineal patterns had no lateral ridges and the lateral fields were marked by irregularities in the striae. This differs from *Meloidogyne arenaria* whose lateral ridges are absent but has short, irregular and forked striae in the lateral fields. This description also differed with that of *Meloidogyne incognita* which has smooth to wavy, sometimes zigzagged striae while in some cases the lateral field is marked by breaks and forks in the striae.

This description did not fit that of *Meloidogyne javanica* which has striae that are smooth to slightly wavy, and some striae may bend toward the vulval edges.

These traits are consistent with *Meloidogyne hapla* (Eisenback, 1981). These findings confirm presence of root knot nematodes in chilli in Nakuru County.

5.3 Effectiveness of sodom apple against root knot nematode disease in chilli

In this experiment, the effect of different treatments of sodom apple fruit extracts was found to be significant. The active compound in sodom apple is solanin. In the greenhouse experiment, during the first reading, high mean heights of the positive control would be attributed to proficient growth as a result of low nematode infestation. The 50% treatment also had high mean heights probably due to low galling of chilli plants. The positive and negative treatment had a high number of leaves probably due to a better rooting system with fewer galls. The mean leaf numbers were not significantly different. This would be attributed to non-effect of the nematode population on nitrogen uptake. Nitrogen levels have been found to have significant effect on the total plant weight including number of leaves. Increased nitrogen levels increased the total number of leaves (Mauyo *et al.,* 2008). The large stem diameters in the positive treatment and 50% treatment could be attributed to better water and nutrient uptake due to healthier roots.

In the field experiment the 100% treatment and 50% treatment had the highest mean height. This is probably due to the fact that the sodom apple eradicated a large number of nematodes leading to better proficient growth. The fact that the 25% treatment had a general low mean height could be attributed to the fact that the treatment was more dilute than the 100% and 50% treatment. The means of leaf numbers were only significant during the first reading. High means of leaf numbers in the 50% treatment could be attributed to better water and nutrient uptake due to reduced nematode population. Nematodes have been known to affect water and nutrient uptake due to vascular dysfunction (Hussey and Junssen, 2002).

The 25% treatment had the lowest number of leaves; this would be attributed to poor water and nutrient uptake due to more deformed roots caused by a higher nematode population. A good vascular system would lead to better nitrogen uptake. Increased nitrogen levels were found to increase the total number of leaves (Mauyo *et al.*, 2008).

During the first reading, the presence of a high number of galls in the positive control treatment would be due to low activity because all treatments were applied once. The activity of the nematicide was more pronounced in the second reading where the galling in the positive control treatment had the second lowest mean. The low number of galls in the 25% treatment could be an indication that it was the optimum concentration for nematicidal activity. This finding is consistent with earlier studies that found larval penetration of second stage juveniles of *M. incognita* was inhibited at various concentrations of leaf extracts and dip durations (Muthamia, 2004).

The efficacy of root-dip treatment with respect to improvement in plant weight and reduction in root-knot development and nematode populations increases with increase in the concentration of leaf extracts and dip durations (Tiyagi and Shamim, 2003).

Earlier studies showed that root knot nematodes have complex trophic relationships with their host plant with induction of specialized feeding structures known as giant cells. Infected plants were observed to grow poorly or even die because of vascular dysfunction (Hussey and Janssen, 2002). The low stem diameter in the 12.5% treatment could be attributed to low concentration of the active compound leading to more root deformation which led to thinner stems due to poor water and mineral uptake. The high number of galls in the negative control treatment would be attributed to the lack of any nematicidal activity which led to increased root distortion. The fact that the 100% treatment had high number of galls could be an indication it was not effective. This would be attributed to its high viscous state which could have resulted to cramping in the soil. The effectiveness of plant extracts varies with the concentration (Muthamia, 2004).

These findings are consistent with previous studies. Reduction in the nematode population is attributed to increased concentration of various substances like ammonia, formaldehydephenol, organic acids, hydrogen sulfide, tannins, and volatile fatty acids which suppress the nematode multiplication and gall formation (Wang *et al*., 2004). In an earlier study leaf extracts of *S. xanthocarpum* inhibits root-knot development in case of root-knot nematodes as observed in egg hatchability and larval mortality tests (Azhagumurugan *et al.*, 2014).

There was significant reduction in the root-knot development caused by *M. incognita*, multiplication of nematode populations of *R. reniformis* and *T. brassicae* on test plants (Muthamia, 2004). Neem based nematicides are known to control root knot nematodes in chilli (Javed *et al*., 2008).

The findings on the effectiveness of sodom apple fruit extracts in the control of root knot nematodes in this research provides a potential source of information that can be used in control of root knot nematodes.

5.4 Effectiveness of *Trichoderma viride* against root knot nematodes in chilli

The lack of significant difference in the means of heights, stem diameters, leaf numbers and gall numbers would attributed be to lack of sufficient chitinolytic enzyme activity. Previous studies have shown *Trichoderma harzianum* significantly reduces the soil population of *Pratylenchus*, *Xiphinema* and *Meloidogyne* in sesame. It has been reported as an effective bio-control agent against root knot and other nematodes (Parveen *et al*., 1993; Saifullah and Thomas, 1996; Khan and Saxena, 1997; Hafeez *et al*., 2000 and Sharon *et al*., 2001). *Trichoderma viride* has been shown to have high rates of mycoparasitism such as that observed against *Meloidogyne incognita.* It is expressed in aggressive nature of isolates against the female body and egg masses. *Trichoderma* species (*Trichoderma atroviridae*, *Trichoderma harzianum*, *Trichoderma rossicum*, *Trichoderma tomentosum*, *Trichoderma virens* have been used to control plant-parasitic nematodes (Hafeez *et al*., 2000). *Trichoderma* chitinolytic enzyme systems play an important role in egg-parasitism. Significant reduction is observed in the root-knot development caused by *M. incognita*.

Our experiments suggest that the chitinase activity of *T.viride* was not very high to cause a significant effect on the parameters measured. It is widely known that environmental parameters such as soil type, soil temperature, soil pH, water potential and biotic factors such as plant species, variety, microbial activity of the soil as well as other factors such as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates (Behzda *et al.,* 2008). Biological control methods are recommended for disease control due to their little negative environmental impact. Organisms used in bio-control also propagate naturally in the environment so they can be replenished naturally (Poornima, 2009).

Trichoderma spp multiplies easily in soil medium, antagonistic strains would therefore be a natural biological control for soil and root knot nematodes (Behzda *et al,* 2008)**.** In vitro monoculture growth rate tests, dual confrontation assays and comparison of strain specific egg-parasitic index (EPI) show that *T. harzianum* strains possess the strongest egg-parasitic ability (Parveen *et al*., 1993).

5.5 Nitrogen and phosphorous determination

In the *Trichoderma* greenhouse experiment, lack of significant effect on nitrogen and phosphorous levels could be as a result of low disease severity (Muthamia, 2004). High nitrogen levels in the negative control and 12.5% treatment during the first reading of the *Trichoderma* extract field experiment could be attributed to better leaf proliferation. This would be a result of low disease severity in the negative control and higher nematicidal activity of the *Trichoderma viride* extract in the 12.5% treatment. The lack of significant effect on nitrogen levels during the second reading could be as a result of low disease severity. High phosphorous levels in the negative control and 100 % treatment during the first reading of the *Trichoderma* extract field experiment could be attributed to better root proliferation. This would be a result of low disease severity in the negative control and higher Snematicidal activity of the *Trichoderma viride* extract in the 100 % treatment.

High nitrogen levels in the 12.5% and 100% treatment during the first reading in the sodom apple fruit greenhouse experiment could be as a result of low disease severity due to increased nematicidal effect (Hussey and Janssen, 2002). Lack of significant difference in nitrogen levels during the second reading and phosphorous levels for both readings are a possible indication of low disease severity. Previous reports indicate phosphorous fertilizers are known to reduce disease severity (Caveness and Ogunfowora, 1985). In the sodom apple fruit extract field experiment, the relatively high nitrogen and phosphorous levels in the 100% treatment could be attributed higher nematicidal activity which led to well-developed roots. Roots attacked by root knot nematodes exhibit characteristic root galls, and infected plants grow poorly or even die because of poor nutrient intake and vascular dysfunction. There was no significant difference in phosphorous levels in the sodom apple fruit extract field experiment. The lack of significance in phosphorous uptake could be as a result of variability of phosphorous which is dependent on environmental factors and plant root characteristics. This finding is consistent with findings by Muthamia (2004).

He found that lack of significance in nitrogen uptake when root knot nematodes were introduced in cow peas was as a result of low disease severity.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. *Meloidogyne hapla* is the most prevalent root knot nematode associated with chilli in Nakuru County.

2. The fruit extracts of sodom apple had significant effect on various growth parameters in chilli. The sodom apple fruit extracts had the highest nematicidal effect and can be used as a more effective control of root knot nematodes.

3. *Trichoderma viride* extracts did not have high nematicidal effect against root knot nematodes.

4. Nitrogen and phosphorous levels in chilli were improved when sodom apple fruit and *Trichoderma viride* extracts were applied against root knot nematodes.

6.2 Recommendations

1. It is recommended that molecular characterisation should be done to determine the root knot nematode species infecting chilli.

2. The active ingredient of sodom apple should be identified. Further research should be done to find out whether the maturity of the fruits has an effect on the nematicidal properties. It is also recommended that sodom apple plants from different ecological zones should be screened to ascertain their nematicidal properties. Further research should be done on the use of sodom apple fruit extracts against root knot nematodes so as to produce a commercial nematicide. More plants known to have medicinal properties should be evaluated for nematicidal activity. Development of root knot nematode resistant chilli cultivars is also recommended.

3. Various *Trichoderma* spp. isolates should be evaluated for nematicidal activity.

4. Nitrogen and phosphorous based fertilizers should be recommended to farmers to reduce the disease severity of root knot nematodes.

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APPENDICES

Appendix 1: Mean square table for heights and leaf numbers in the sodom apple field experiment

<0.05 NS

Sourcce of var	Df	Height 1	Height 2	Height3	Leaf 1	Leaf 2	Leaf 3
Reps	2	0.7222NS	3.1667 NS	9.5 NS	91.5NS	1.3889NS	443.1677NS
Treatments	5	9.1222 NS	13.2 NS	17.3NS	34.2667 NS	67.5222NS	317.167NS
Cv		16.0466	12.4178	11.53	40.4157	27.1243	36.1061
Mean		13.0556	19	22.33	19	27.0556	38.5

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 2: Mean square table for stem diameters and number of galls in the sodom apple field experiment

<0.05 NS

Sourcce of var	Df	Diam 1	Diam 2	Diam 3	Gall 1	Gall 2
Reps	2	0.4444 NS	0.2222NS	0.3889 NS	11.0556NS	171.167S
Treatments	5	0.2778 NS	0.0556NS	0.5889NS	17.5222NS	82.6333NS
Cv		23.5969	22.8364	18.6909	49.159	39.2663
Mean		2.2778	2.6111	3.6111	4.7222	13.5

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 3: Mean square table for N/P levels in the sodom apple field experiment
<0.05 NS

Sourcce of var	Df	N 1	N 2	P1	P2
Reps	2	0.6274 NS	0.1589NS	1608837NS	1503325NS
Treatments	5	2.9805 NS	1.4770NS	5505094NS	2870946NS
Cv		45.1916	66.7156	147.528	91.7335
Mean		1.9713	1.8439	1209.58	2265.67

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 4: Mean square table for heights and leaf numbers in the sodom apple greenhouse experiment
<0.05 NS

Sourcce of var	Df	Height 1	Height 2	Height3	Leaf 1	Leaf 2	Leaf 3
Reps	2	30.722S	235.5S	316.1667NS	33.722S	154.17NS	117.0556NS
Treatments	5	6.7556NS	30NS	91.7NS	15.156NS	43.967NS	28.0889NS
Cv		15.74	37.986	42.713	23.873	35.211	38.223
Mean		10.222	17	21.8333	11.889	18.5	16.111

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 5: Mean square table for stem diameters and number of galls in the sodom apple greenhouse experiment
<0.05 NS

Sourcce of var	df	Diam 1	Diam 2	Diam 3	Gall 1	Gall 2
Reps	2	0.7222S	0.7222S	0.7222NS	18.7222NS	97.2222NS
Treatments	5	0.1889NS	0.2222NS	0.3556NS	5.2556NS	33.9556NS
Cv		20.3	18.508	22.212	66.687	97.039
Mean		1.7222	1.8889	2.5556SS	4.6111	5.8889

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 6: Mean square table for N/P levels in the sodom apple greenhouse experiment

<0.05 NS

Sourcce of var	df	N 1	N 2	P1	P2
Reps	2	0.0817NS	0.00761NS	367141NS	954138NS
Treatments	5	0.1191NS	0.02542NS	295977NS	468132NS
Cv		18.7494	14.1036	111.079	97.0324
Mean		1.0433	1.0844	791.203	877.593

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 7: Mean square table for heights and leaf numbers in the *Trichoderma viride* field experiment

<0.05 NS

Sourcce of var	Df	Height 1	Height 2	Height3	Leaf 1	Leaf 2	Leaf 3
Reps	2	1.5NS	0.1667NS	65.722NS	5.1667NS	18.389NS	507.06NS
treatments	5	2.6667NS	3.2NS	39.522NS	31.567NS	25.789NS	209.29NS
Cv		21.995	16.373	26.674	31.607	31.508	47.838
Mean		12	17.667	24.389	18.833	25.056	36.222

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 8: Mean square table for stem diameters and number of galls in the *Trichoderma viride* field experiment
<0.05 NS

Sourcce of var	df	Diam 1	Diam 2	Diam 3	Gall 1	Gall 2
Reps	2	0.2222NS	0.1667NS	0.0556NS	11.556NS	897.56NS
Treatments	5	0.0889NS	0S	0.3222NS	5.3889NS	656.22NS
Cv		14.123	22.707	18.691	65.141	80.434
Mean		2.1111	2.667	3.611	4.6111	29.889

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 9: Mean square table for N/P levels in the *Trichoderma viride* field experiment
<0.05 NS

Sourcce of var	df	N 1	N 2	P1	P2
Reps	2	1.1483NS	0.6149NS	112.97NS	7E+0.6
Treatments	5	1.3202NS	0.6355NS	479703S	3E+0.6
Cv		54.267	60.586	75.37	91.411
Mean		1.54	1.4672	475.28	1684.4

Appendix 10: Mean square table for heights and leaf numbers in the *Trichoderma viride* greenhouse experiment
<0.05 NS

Sourcce of var	Df	Height 1	Height 2	Height3	Leaf 1	Leaf 2	Leaf 3
Reps	2	6.8889NS	187.056S	56.1667NS	22.8889NS	140.056NS	2.6667NS
Treatments	5	8.1889NS	12.4889	47.4333NS	19.5556NS	17.2556NS	21.333NS
Cv		23.8817	36.8841	31.7218	37.0767	42.7766	35.2777
Mean		10.3889	16.4444	16.1667	12.4444	19.3889	16.3333

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 11: Mean square table for stem diameters and number of galls in the *Trichoderma viride* greenhouse experiment

<0.05 NS

Sourcce of var	df	Diam 1	Diam 2	Diam 3	Gall 1	Gall 2
Reps	2	0.5S	0.3889NS	0.5NS	54.0556NS	167.056NS
Treatments	5	0.1NS	0.1889NS	0.1NS	6.6222NS	45.6889NS
Cv		17.2488	21.1615	21.9089	68.5613	103.636
Mean		1.83333	2.3889	5.5	6.4444	8.4444

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different

Appendix 12: Mean square table for N/P levels in the *Trichoderma viride* greenhouse experiment

<0.05 NS

Sourcce of var	df	N 1	N 2	P1	P2
Reps	2	0.00906 NS	0.00187NS	1785040NS	6866.86NS
Treatments	5	0.04319 NS	0.00501NS	521907NS	343576NS
Cv		13.2242	8.2439	85.8816	119.227
Mean		1.09059	1.13222	837.941	806.76

Df- Degrees of freedom

Cv- Coefficient of variation

NS- Not significantly different

S- Significantly different