MANAGEMENT OF POTATO CYST NEMATODES USING HOST RESISTANCE, ORGANIC AMENDMENTS AND BIOCONTROL AGENTS IN NYANDARUA COUNTY, KENYA

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This thesis is my original work and has not been presented for the award of a degree in any other University

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To Pamela Odero and my siblings

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ACRONYMS AND ABBREVIATIONS

ANOVA: Analysis of Variance

DAP: Diammonium Phosphate fertilizer

EPPO: European and Mediterranean Plant Protection Organization

F.A.O: Food and Agricultural Organization of United Nations

ICIPE: International Centre of Insect Physiology and Ecology

J2: Second Stage Juveniles

KALRO: Kenya Agricultural and Livestock Research Organization

KBL: Kenya Biologics Limited

MOA: Ministry of Agriculture

NaOCl: Sodium hypochlorite

NPCK: National Potato Council of Kenya

PCN: Potato Cyst Nematodes

BCAs: Bio-Control Agents

Pi; Initial population

Pf; Final population

GENERAL ABSTRACT

Globally, potato cyst nematodes species (*Globodera pallida* and *Globodera rostochiensis*) are characterized by their invasive nature, efficient adaptability in most environments, and vast spread in potato fields. Given the invasive potential of this pest, its mitigation entails restricting the movement of soil, planting materials, and farm tools in established infested farms. Therefore, the study aims at determining the level of resistance in local potato cultivars, efficacies of organic amendments and bio-control agents (BCAs) in managing potato cyst nematodes. In achieving these objectives, experiments were conducted in the laboratory, greenhouse, and open field already infested with potato cyst nematodes. Laboratory and greenhouse experiments were conducted at Upper Kabete, University of Nairobi whereas the subsequent field experiments were conducted in selected farms in Nyandarua County. To determine the level of resistance of locally available potato cultivars to potato cyst nematodes, the following potato cultivars were used; Dutch Robijn, Shangi, Roslin Tana, Tigoni, Asante, Sherekea, Nyota, Unica, Chulu, Arka, Kenya Mpya, Desiree and Manito. Potato cultivar 'Shangi' was used to assess the efficacies of organic amendments (chicken, pig, cow, goat and green manure) and bio-control agents; *Purpureocillium lilacinum, Trichoderma asperellum, Trichoderma atroviride, Trichoderma hamatum, Trichoderma harzianum* and *Bacillus subtillis* on potato cyst nematodes, respectively. Data collected from the experiments was subjected to analysis of variance and their means separated using Tukeys multiple comparisons ($p<0.05$). Potato cultivars Sherekea and Nyota were considered partially resistant to PCN with a severity score of 4-6 (<25-3%) whereas cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana were susceptible with a severity score of 1-3(>100-5%). The reproductive index of PCN viable eggs of all the 11 potato cultivars either susceptible or partially resistant were < 1 compared to the positive control potato cultivar Desiree.

For all organic amendments, potato cyst nematode count was reduced by 50% under greenhouse whereas the reduction was 85% with a reproductive index of <1 for cow, goat and green manure compared to the control in the open field. All commercial BCAs had < 25 potato cyst nematode count compared to the control which had 48-53 cysts under the greenhouse. In the open field, the BCAs namely *T. asperellum*, *T. atroviride* and *P.lilacinus*, had < 23 final cysts count from an initial count of 106-247 cysts in the first season whereas the second season had < 7 final cyst count from an initial count of 19-166 cysts across cultivars Desiree, Shangi and Markies. This study established two tolerant potato cultivars; Nyota and Sherekea. Additionally, all bio-control agents except *Bacillus subtillis* and organic amendments were effective in suppressing potato cyst nematodes population densities. Therefore, integration of tolerant potato cultivars, bio-control agents and organic amendments can be recommended for sustainable management of potato cyst nematodes.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum* L.) is a major food and cash crop that is grown by over 800,000 small-scale farmers in the Kenyan highlands. Owing to its significance to food security and the economy, close to 120,000 hectares of land in Kenya is under potatoes with a potential average yield of 7.7 t/ha annually (Muthoni *et al.,* 2013). In these Kenyan highlands, potato is highly prioritized over maize as a source of energy and protein (Devaux *et al.,* 2020). Potato is second to maize in the ranking of the most widely consumed food crop in Kenya and it provides higher yields on less land than any other cash crop (Birch *et al.,* 2012; Devaux *et al.,* 2014). It serves as food as well as a cash crop for semi-urban and rural dwellers thus playing a key role in food security and income generation (Janssens, 2013; Devaux *et al.,* 2020). Potato consumption in Kenya has grown from production of about 2.3 million to 2.9 million tones recording a 9% increase from the year 2009 to the year 2013, respectively (Janssens, 2013).

Over 60% of the fresh potato produce is channeled to urban consumers and absorbed through fast food restaurants, and market stalls (Van Der Lans *et al.,* 2012). High-quality potatoes are preferred for processing but remain a challenge among farmers due to high pests and disease pressures (Mburu *et al.,* 2020). The main potato-growing areas in Kenya are counties within the Mt Kenya region (Kimani *et al.,* 2015). The other potato-growing regions include Mau Narok, Molo, Tinderet, Nandi escarpments, Cheranganyi hills, Kericho, Kisii, and Taita Taveta (Okeyo *et al.,* 2017). Besides, potato production faces challenges such as lack of clean seeds, adverse weather conditions occasioned by two bi-modal rainfalls, ineffective approaches for managing pests and disease, lack of a proper marketing-oriented system, and ineffective packaging policies (Janssens, 2013).

The two invasive and fast-spreading potato cyst nematode species *Globodera pallida* and *Globodera rostochiensis* were first reported in 2019 (Mburu *et al.*, 2020) and 2015 (Mwangi *et al.*, 2015), respectively in Nyandarua county. Apart from its first detection in Nyandarua County, over 20 counties growing potatoes have reported potato cyst nematode infestation, hence posing a significant threat, especially to potato seed production in Kenya (Mburu *et al.*, 2020). Unlike other pests, potato cyst nematode is featured with a well-established pathogenic ability to survive for over 20 years without a suitable host in the field (EPPO, 2013). In a survey to establish the level of infestation of potato cyst nematodes conducted in 20 potato-growing counties in Kenya, a prevalence of 80 to 100% was recorded in Nyandarua, Elgeyo Marakwet, Nakuru and Narok being severely infested (Mburu *et al.*, 2018). Moreover, Mwangi *et al.* (2015), had shown yield losses of up to 80% in Kenya due to potato cysts nematodes. Despite this imminent threat to potato production, management approaches against potato cyst nematodes remain elusive (Ochola *et al.*, 2021).

Problem statement

Potato cyst nematodes are an economically important pest of potatoes with strict quarantine regulations in the world (EPPO 2013). Potato cyst nematode (*Globodera* spp) feed on potato roots and of other Solanaceae families such as tomato and black nightshade plants (Coyne *et al.*, 2018; Niere and Karuri, 2018). As a serious pest to potatoes, its dissemination through wind, rain, water, soil, vehicles and plant material can cause complete crop failure if not quarantined and controlled once it occurs in the area (Castelli *et al.*, 2006).

Upon potato cyst nematode infestation, potato plant shows above-ground symptoms i.e. partial leaf wilting, discoloration and below ground symptoms i.e. presence of cysts on roots that reduce the root mass. The other symptoms of potato cyst nematodes are stunted growth, early senescence leading to an estimated 80% yield loss characterized by unmarketable-sized potato tubers (Castelli *et al.*, 2006). The ability of females of potato cyst nematodes to survive for over 20 years in the soil is attributed to the quiescent hardened body structure- cyst- that measures ~0.5 mm in diameter enclosed with 300 to 500 eggs (EPPO, 2013). These factors complicate their management, hence necessitating research in multifaceted approaches (Ochola *et al.*, 2021).

Farmers in potato growing areas through the initiative of the ministry of agriculture, county governments have been managing the pest through crop rotation with non-Solanaceae crops, trap cropping and nematicides. However, potato cyst nematodes (PCN) have no commercial nematicides and tolerant cultivars (Trudgill *et al.*, 2014). Therefore, integrated management of PCN for adoption by small-scale farmers needs further studies to determine tolerant potato varieties, bio-control agents and organic amendments. Since potato cyst nematode is a recently reported pest in Kenya, there is a dearth of information on its management using organic amendments, resistant varieties and biological control agents.

Justification

Potato cyst nematode (PCN) is a newly introduced invasive pest of potato which originated from the Andes Mountains in South America and was first detected in Kenya in 2015. Resistant/tolerant cultivars have been successfully deployed to reduce yield loss in European countries. However, the levels of resistance or tolerance to potato cyst nematodes in varieties grown in Kenya are yet to be established. Suppression of plant parasitic nematodes using organic amendments has been demonstrated by several studies i.e. Karmani *et al.*, 2011 and Tanimola and Akarekor, 2014 showed that amending the soil with animal manure successfully controls root-knot nematodes. Given that small-scale farmers commonly use organic amendments, their potential in managing potato cyst nematodes would be an added advantage. Biological agents are considered among sustainable strategies for managing plant-parasitic nematodes. In addition, bio-control agents have a site-specific functionality influenced by the environment. This study aims at developing an integrated package for managing potato cyst nematodes comprising of resistant or tolerant potato cultivars, bio-control agents (fungal/bacterial bio-control products) and organic amendments, which will be of help to small-scale farmers and seed producers in the production of potato cyst free tubers and healthy tubers.

1.2 Objectives

1.2.1 Broad objectives

The general objective of this study was to increase productivity and enhance resilience in potato production against the backdrop of climate change and variability

1.2.2 Specific objectives

I. To identify high-yielding potato cultivars with resistance/tolerance to potato cyst nematodes

II. To determine the suppressive potential of organic amendments to potato cyst nematode

III. To determine the efficacy of bio-control agents in the management of potato cyst nematode

1.3 Hypothesis

I. Potato cultivars grown in Kenya are susceptible to potato cyst nematodes

II. Organic amendments have no suppressive effect on potato cyst nematodes

III. Bio-control agents are not effective in suppressing potato cyst nematode

CHAPTER TWO

LITERATURE REVIEW

2.1 History and origin of potato

Potato (*Solanum tuberosum*), was first domesticated by the native Americans and later introduced in multiple locations in present-day Peru (Zhang *et al*., 2010). By the 2nd half of the 16th century, potato was cultivated in Europe after its introduction from America by Spanish traders (Singh *et al*., 2014). Irish potato was introduced and cultivated in Africa around the 20th century, and since, its production has increased from 2 million tons in 1960 to 16.7 million tons in 2007 (Olanya *et al*., 2012). In the 1880s, British farmers introduced potatoes to East Africa (Rodger. 2007).

Globally, Andigena or Andean and Tuberosum or Chilean, are the major cultivated subspecies of *Solanum tuberosum* (Spooner *et al.,* 2006). In Kenya, Kenya Agricultural and Livestock Research Organization (KALRO) have introduced high-yielding varieties namely Shangi, Sherekea, Kenya Mpya and Tigoni having the potential of producing 160 bags/ acre (Muthoni *et al.,* 2013). Moreover, these varieties mainly do well in Nyandarua, Bomet, Meru, Uasin Gishu, Nakuru, Kiambu, Nyeri, Narok, West Pokot and Keiyo Marakwet counties that are characterized by wet climatic conditions with slightly acidic soil (Kimani *et al.,* 2015). Early maturing potato cultivars in Kenya are; Annett, Kerr's Pink, Kenya Chaguo, Roseline Tana, Kenya Dhamana, Tigoni and Asante. Medium-maturing potato varieties in Kenya are Shangi, Caruso and Connect whereas late-maturing varieties are Purple gold and Rudolph (Muthoni *et al.,* 2016). Potato cultivars Spunta, Manitou and Markies are resistant to potato cyst nematodes (Muthoni *et al.,* 2016). Unfortunately, these varieties are in a vague of extinction, hence extensive seed bulking practices are necessary to salvage the varieties for further studies as well as adopted to manage PCN (Mburu *et al.,* 2020).

2.2 Botany of potato

Potato is in the phylum; Anthophyta, class; Magnoliopsida, order; Solanales, family; Solanaceae, genus; *Solanum*, species; *Solanum tuberosum* (Kui *et al*., 2022). The family Solanaceae has 2000 other species such as tomato (*S. Lycopersicum* L.), sweet pepper (*Capsicum annuum* L.), eggplant (*S. melogena* L. var. *esculentum*), tobacco (*Nicotiana tabacum* L.) and petunia *petunia hybrid* L. (Seguí-Simarro *et al.,* 2016). The genus *Solanum* has been divided into several sections. For instance, *S. tuberosum* that belong to the section petota has about 200 wild species which grows in a range of habitat such as semi-desert, sub-tropical and temperate regions at an altitude of between 1200 to 3800m (Seguí-Simarro *et al.,* 2016).

Potato (*Solanum tuberosum*) is a perennial crop that can grow up to 60 cm, it has long leaves which are pinnate in shape and with white, yellow, blue, pink and purple flower pigmentation arranged in clusters (Reddy *et al.,* 2018). Upon flowering, potato plants usually produce inedible seedless green cherry tomatoes fruits which have toxic alkaloid solanine, mature potatoes produce edible underground tubers that reserve food for utilization by the plant during the vegetative propagation stage, especially in cold seasons (Spooner *et al.,* 2012). Additionally, the edible underground tubers, which are also the plant propagules, develop eyes/buds on the surface which sprout to produce new shoots under conducive conditions (Navarre *et al.,* 2014).

2.3 Potato production

Between 1966 and the 1980s, before the world experienced the green revolution, potato production acreage in North America and west Europe was annually reduced by more than 2% while yield had a 1% increase (Van der zaag *et al.,* 2000). In the same period, acreage of land under potato production in Asia grew by 7%, followed by an annual yield of 2%, whereas in Africa there was a 4% increase in the acreage of land under potato production though the yield of potatoes remained constant during this period (Van der zaag *et al.,* 2000). Whereas North America and West Europe potato production are 40 t/ha, Africa's production is less than 20 t/ha (Gildemacher, 2012). Potato production in Kenya serves as an earning stream to about 2.5 million people across the potato production value chain with between 500,000 to 1 million farmers involved in potato production/farming (Abong and Kabira, 2013). Kenyan Ministry of agriculture classified potatoes under "orphan crop" due to the low level of development even though more land has been devoted to the crop (NPCK, 2019).

2.4 Potato production constraints

Production of potatoes in Kenya is majorly constrained by pests, diseases, poor agronomic practices and soil fertility, seasonality in potato production, inadequate or no certified seeds, high cost of inputs and marketing constraints (Jane. 2009). The complexity of these challenges has been related in several studies to negatively impact on growth, yields and acreage of potatoes not only in Kenya (Muthoni *et al.,* 2013). Among the important pests of potatoes are green peach aphids (*Myzus persicae*), cutworm (*Agrotis* spp), epilachna beetles (*Epilachna* spp), potato tuber moth *Pthorimaea operculella* (Radcliffe *et al.,* 2007).

Potato diseases caused by viruses, bacteria and fungi reduce the quality and quantity of tuber yield. Potato leaf roll virus (PLRV) and potato virus Y, are among the viral diseases whereas bacterial canker (*Clavibacter michiganense* subspp *michiganense*) is a bacterial disease (Jane, 2009; Hutton *et al.,* 2015). Fungal diseases of potatoes include; late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*), leaf spot (*Botrytis cinerea*) and fusarium wilt *Fusarium oxysporum* (Zaker *et al.,* 2010; Okungbowa *et al.,* 2012; Kemmitt., *et al.,* 2013; Sun *et al.,* 2016).

Potato production in tropical soil with low organic matter and nutrient imbalance delays potato tuber initiation and growth (Wekesa *et al*., 2014). For instance, higher nitrogen application results in higher plant canopy growth with low tuber formation (Lynch *et al.,* 2012). The response of potato plants to N fertilization in the soil depends on the potato variety, soil type and rainfall pattern during the plant growth (Ospina *et al.*, 2014).

High soil salinity is associated with leave senescence, reduced tuber size and yield per plant in potato production (Dahal *et al.*, 2019). During the rainy season, the plant is normally rain-fed and water stored in the soil, while during the low rainfall season, the capacity of soil to store water is critical and water supply below 60% leads to water stress (Badr *et al.,* 2012). High tropical temperatures reduce overall potato growth and tuber yield (Raymundo *et al.*, 2018). High light intensity increases photosynthesis, stimulates flowering, initiates tuber growth and early leaves senescence. Conversely, low light intensity increases top plant growth, reduces tuber weight and plant dry matter content and changes plant morphology (Xiao *et al.*, 2011).

2.5 Economic importance of potatoes

Potato tubers contain essential minerals and vitamins such as potassium, vitamin C, vitamin B6 and folate (Navarre *et al*., 2009). Besides being an income earner for most small-scale farmers its use in various forms cut across local and international as well as for home utility and manufacturing industries (Ugonna *et al.,* 2013). Several recipes of potatoes among consumers, (French fries from frozen potatoes) are widely distributed in most fast-food restaurants in Kenya and the world at large (Tesfaye, 2010). Dehydrated potato flakes, as a product of mashed potato and an ingredient in potato snacks, are used as a food aid (Pedreschi, 2009).

Potato flour has been extensively adopted for binding mixtures of meat and thickening gravies, soups, sources, stew and as a binding agent in bakeries (Himashree *et al.,* 2022). Industries handling pharmaceutical, textile, wood and paper employ adhesive, binder and texture agents from potato starch as well as production of fuel-grade ethanol from potato peels (Ugonna *et al*., 2013).

Potatoes have starch, which is converted to fermentable sugars when crushed and heated then used in the distillation of alcoholic beverages (Ugonna *et al*., 2013).

2.6 Potato cyst nematodes (*Globodera* spp)

2.6.1 Taxonomy of potato cyst nematodes

Potato cyst nematode belongs to the kingdom; Animalia, phylum; Nematoda, class; Nemata, class; Secernentea order: Tylenchida, suborder; Tylenchina, family; Heteroderidae, Genus; *Globodera* (Wainer *et al*., 2021). Two closely related potato cyst nematode species, *Globodera rostochiensis* and *Globodera pallida*, are of economic importance to potato production in Kenya and the world (Mwangi *et al.,* 2015).

2.6.2 Distribution of potato cyst nematodes

In 1600, the export of potatoes from South America to the rest of the world, acted as a source of dissemination of *Globodera rostochiensis* (golden cyst nematode) and *Globodera pallida* (white cyst nematode) as a common pest, though the place of origin of the two pests remains elusive (Oro *et al.,* 2014). Further and continuous dissemination of potato cyst nematodes to over 100 countries in the world, elevated potato cyst nematodes current situation to become biosecurity and a quarantine concern (EPPO. 2013). In Europe, PCN presents a threat to food security owing to its establishment and immeasurable damages, hence mitigated through screening and restricting the movement of PCN-infested potato plants in European border countries (EFSA Panel on Plant Health, 2012).

The occurrence and increase in the population of PCN in already established zones and new areas have an imminent risk to potato production if economically justifiable strategies are not imposed and implemented (Abd-Elgawad *et al.,* 2015). Before 1972, PCN was introduced in New Zealand while in Australia it was detected near Perth in 1986 with *G.rostochiensis* being the most dominant

PCN species found (Faggian *et al*., 2012). In Kenya, *G. rostochiensis* and *G. pallida*, the white cyst nematode was first reported in 2015 and 2019, respectively (Mwangi *et al*., 2015; Mburu *et al*., 2020). Within the temperate regions of the world, *Globodera rostochiensis* is common among potatoes, tomatoes and eggplants with potatoes (*Solanum tuberosum*) being the major host (EPPO, 2013).

2.6.3 Morphological characteristics of *Globodera* spp

Males of *Globodera rostochiensis* (golden cyst nematodes) are morphologically vermiform shaped with short tails without bursa. Moreover, *Globodera rostochiensis* is 0.89-1.27 mm in length and width of 28 µm. However, they have a curved body with a posterior region twisted at an angle of 90º to the rest of the body upon fixation. On the contrary, females of *Globodera rostochiensis* form cysts from a hardened dead cuticle characterized by an intact vulval basin (Wainer *et al.*, 2021). Aged cysts in the soil tend to lack signs of genitalia except for a hole in the cuticle to determine the fenestral basin whereby its length without neck measured 445 µm; width 382 µm (Wainer *et al.*, 2021). A vermiform *Globodera rostochiensis*-second stage-juvenile- has a visible tapered tail and a round head, which is about two-thirds of its entire length (EPPO, 2013; Wainer *et al.*, 2021). Previous studies show the stylet of *G. rostochiensis* being less than 30 µm and further characterized by a lateral field with four lines and esophageal glands around the body cavity (EPPO. 2013; Wainer *et al.*, 2021).

Males of *Globodera pallida* are pale with a vermiform shape seen from their C or S shape twist with distinct four lateral field lines and a short tail, the spheroidal-shaped females have short necks without a terminal cone that forms a cyst (Wainer *et al.*, 2021). Furthermore, it has a thick cuticle with a superficial lace-like pattern with the anus and vulva lying in a vulval basin (EPPO, 2013; Wainer *et al.*, 2021).

2.6.4 Infection processes of potato cyst nematodes in its host

The second-stage juveniles (J2) of PCN hatch from eggs upon stimulations from host root exudates in the soil and subsequently invade the roots (Bell *et al.,* 2021). Numerous (J2) prefer feeding on the pericycle, cortex, or endodermis cells that further become feeding tubes (Castelli *et al.,* 2006). The J2 completes two juvenile stages as they mature into adults with varying sex inside the roots of the host plant (Price *et al.,* 2021). Adult female swells and breaks through the root surface but remain attached to the root of the host plant (Ochola *et al.,* 2021).

Moving males fertilize the females before they die whereas females remain attached to the roots (Ochola *et al.,* 2021). Females of potato cyst nematode species are white when protruding from the root surface. However, they part in color whereby *Globodera rostochiensis* females became golden yellow for 4-6 weeks while *Globodera pallida* remain white (EPPO, 2013). Mature females die having hard skin that turns brown to shield the eggs within the cyst that will hatch for the next generation (EPPO, 2013). Upon the death of female potato cyst nematodes, cysts have an average of 500 eggs detached from the roots and fall to the soil. As for the eggs, they can hatch immediately or wait for the subsequent season to infest host plants (Castelli *et al.,* 2006). Potato cyst nematodes remain dormant for over 20 years without an alternate solanaceous host such as eggplant and tomato. (EPPO 2013; Duceppe *et al.,* 2017).

2.6.5 Edaphic factors favouring potato cyst nematodes multiplication

Globally, Potato cyst nematodes normally co-exist with the host plant and for instance, PCN upon its dissemination in either running water, farm implements, seeds or soil, it punctures the susceptible host roots invading the plant tissues during growth and development in the plant (EPPO 2013). Potato cyst nematodes are well-known to establish well under well-aerated and acidic soil of PH of 5.0 and soil properties which potato grows well in Kenyan highlands (Mburu *et al*., 2020).

Host root exudates and soil water/moisture induce the hatching of the cyst nematode eggs to the second juvenile stage (EPPO, 2017). Temperature range of between 5 to 20 degrees, availability of oxygen, moisture content in the soil and absence of physiological barriers i.e. diapause is suitable for potato cyst nematodes multiplication in the northern hemisphere (Baklawa *et al.*, 2017).

2.6.6 Symptomatology of potato cyst nematode damage

The primary symptoms associated with potato cyst nematode infestation are patches of chlorotic and wilted plants with presence of cyst on the roots (Moens *et al.*, 2018). Yield losses caused by PCN are characterized by small tuber during harvesting and reduced root mass (EPPO, 2013; Castelli *et al*., 2006). In addition, the roots of host plants infested with PCN have golden to white cysts (Trudgill *et al*., 2003). Secondary infestation is exhibited by reduced and poorly grown root systems due to nutrient deficiencies and water stress (Mburu *et al.*, 2020).

2.6.7 Economic importance of potato cyst nematodes

In Europe, close to 9% losses are annually recorded by an infestation of PCN in potato fields, but the pest can be devastating in temperate areas when occasioned by a virulent pathotype on potato cultivars with low resistance (Turner and Subbotin, 2013). Few commercial cultivars show resistance to *Globodera pallida* but have undesirable traits (Mburu *et al.*, 2020). Potato cyst nematodes cause yield loss of up to 80% in potato-growing areas in Kenya and under heavy infestation (Mwangi *et al.*, 2015). Studies have revealed that potato cyst nematodes are a serious threat to potato production in Kenya where an average yield of 9.9 t/ha is realized compared to the potential yield of 40 t/ha which is equivalent to annual losses of $127 million (EPPO, 2013).

2.7 Management of potato cyst nematodes

Potato cyst nematodes is an invasive and first-spreading pest, where it is normally misdiagnosed with soil nutrient deficiency on the farm, with stunted, chlorotic potato plants growing in patches (Nicol *et al.*, 2011). Therefore, its occurrence in an area should be contained under strict quarantine and management of PCN requires intensive and realistic approaches (EPPO 2013). However, cost, availability of arable land, species of the nematode, which have over 2000 species of alternative hosts and abiotic and environmental factors, limit these methods of control such as the use of chemical nematicides, crop rotation with a crop of a different family from the host plant and use of resistant potato varieties (Bennett *et al.*, 2012).

Alternative options available with a lower negative impact on the environment and a wider range of PCN management options have received more attention in recent years (Renčo *et al.*, 2014). These alternatives are as effective as synthetic nematicides since they are readily available, cheaper and safe for the environment (Verginer *et al.*, 2010). Bio fumigation, mycorrhization, plant extracts, essential nematicidal plant oils, nematicidal plant metabolites and organic-waste amendments such as green manure from brassica plants have been successfully used to manage potato cyst nematodes in European countries and some African countries (Renčo *et al.* 2011; Avato *et al*., 2013).

It is important to develop efficient and more effective integrated pest management approaches to manage potato cyst nematodes. Over the past ten years, much effort has been vested in determining control techniques for potato cyst nematodes. Examples of these approaches were trap cropping with *Solanum sisymbriifolium* (Timmermans *et al.*, 2006), use of resistant potato cultivars such as Markies (Castelli *et al.*, 2006) and the use of bio-control agents such as *Pochonia chlamydospores* (Mhatre *et al.*, 2022).

2.7.1 Chemical control

Synthetic nematicides have been widely used in the control of various species of plant parasitic nematodes (PPN) in the world. For instance, fumigation is widely adopted to reduce large potato cyst nematode densities in sandy soil covered with polythene sheeting (Kantharaju and Reddy, 2001). The risk of these synthetic nematicides includes groundwater contamination and since most of their active ingredients are in a class of hazardous chemicals, are in the process of being phased out or already banned due to safety concerns (Ntalli *et al*., 2017).

The application of chemical nematicides in the soil normally acts by delaying the penetration of plant-parasitic nematodes into the roots of the host plant hence making the host plant escape nematode infestation during growth and development (Fatemy, 2018). The application of nematicide is normally on patches found within the field, which is related to the age of infestation and the degree of the colonization of the field by PCN (Evans *et al.,* 2003). Examples of synthetic nematicides are 1, 3 Dichloropropene which is mixed in various quantities with other compounds and Dazomet which releases methyl isothiocyanate (Chakraborty *et al*., 2020). Research studies conducted by (Haydock *et al.,* 2010), on the use of 1,3-D or Oxamyl to control *Meloidogyne chitwood* in potato plants increased ware yield by 21.1t/ha, total yield by 22.8t/ha.

2.7.2 Crop rotation

Crop rotation is frequently used in potato farms to manage potato cyst nematode infestations by planting crops that are of different families to potatoes (Mburu *et al.,* 2020). Crops grown in a single stand for long durations facilitate densities increase of potato cyst nematodes that reduce yields (López-Lima *et al.,* 2013). Pacajes *et al*., (2002), showed that PCN reproductive index was less than 1 when PCN infested field was rotated with beans (non-host crop) for 1 year. The annual decline rate of potato cyst nematodes in the soil is dependent on the non-host used, initial population densities and various soil-related factors (López-Lima *et al.,* 2013). Practicing short crop rotation with a susceptible crop normally results in a distinct increase in plant-parasitic nematodes (root-knot nematodes) in the soil, while potato cyst nematodes in the absence of a host plant, it normally takes 15 years for all the cysts to hatch and die (Christoforou *et al.,* 2014). Crop rotation effectively controls PCN through the integration of resistant cultivars, trap crops, or the application of synthetic nematicides (Turner *et al.,* 2006). Crop rotation, however, requires a large parcel of land for its efficiency (López-Lima *et al.,* 2013).

2.7.3 Trap crops

Trap crops reduce potato cyst nematodes population but crops used for trapping are of low economic value than the host crop (Bélair *et al*., 2016). For instance, *Solanum sisymbriifolium* was grown to stimulate the hatching of potato cyst nematodes eggs to J2 and as a management and uprooted 7 weeks after planting (Dias *et al.,* 2012). *Solanum sisymbriifolium* stimulated 50-80% hatching in eggs, which is slightly lower than that of normal Irish potato (Dias *et al.,* 2012). Nematode densities increased when the crop was destroyed late and with the application of trap cropping, the *Globodera rostochiensis* population was reduced by over 80% (Turner *et al.,* 2006).

Studies on the pathogenicity of four *S. sisymbriifolium* cvs (Domino, Pion, Sis 4004 and Sharp) and five *Meloidogyne* species have proven that *S. sisymbriifolium* is resistant to *M. chitwood* and hyper-susceptible to *M. arenaria* and *M. hapla*. *Meloidogyne hispanica* only cv Pion was found to be susceptible as cv Domino resistant and Sis 4004 hyper susceptible while on the five Meloidogyne species tested, *S. sisymbriifolium* cvs root exudates had hatching inhibition to the second stage juveniles (Dandurand and Knudsen, 2016).

This method is effective against plant-parasitic nematodes compared to, longer crop rotation which results in a decrease in revenue to the farmers, use of resistance which has become ineffective to

PPN due to new virulence group selection by repeated use of partially resistant cultivars (Turner and Fleming 2002; Tzortzakakis *et al*., 2005). Using chemical nematicides has also failed in the reduction of nematode infestation in the soil (Evans and Kerry 2007). A study by Olabiyi *et al*., (2008); Shakil *et al*., (2008) showed efficiency in the control of plant-parasitic nematodes (PPN) by combining trap crops with antagonist plant extracts such as polyphenols to synergistically exert a nematicidal effect.

Trap crops attract plant-parasitic nematodes by root exudations and are immediately uprooted or destroyed before allowing nematodes to complete their reproductive cycles (Scholte, 2000). Over 90% reduction of PCN was observed with the use of the family Solanaceae as trap crops (Scholte. 2000; Timmermans. 2006). Resistant trap crops with a high production level of hatching agent are usually effective as compared to susceptible trap crops when it comes to critical timing of the trap crop destruction, hence an ideal control method for PCN (Scholte. 2000).

2.7.4 Soil solarization

Soil solarization involves the use of two-layered polythene to allow the soil underneath to heat quickly (Ioannou, 2001). Soil solarization has been widely used in the suppression of soil-borne pests as an alternatively environmentally safe method since, during the process of soil solarization, there is an increase in soil temperatures, which causes the death of soil-borne pathogens (Greco *et al.,* 2000). Soil solarization improves potato growth and production as well as reducing the infestation levels in the soil and roots by plant-parasitic nematodes such as *M. javanica* (Oka *et al*., 2007).

A study by D'Addabbo *et al.* (2010), showed a 95% reduction of *Globodera rostochiensis* population after 62 days of solarization but less effective in a cool climate and at soil depths of over 10 cm. On the other hand, in Tunisia, soil solarization on land incorporated with 70 t/ha of cattle manure reduced the population of *M. javanica* by 38.14% (El Hajji *et al.,* 2012). On the contrary, Horrigue-Raouani and B'Chir. (1998), showed that soil solarization increased soil moisture and subsequently enhanced the hatching of eggs of root-knot nematodes as well as increased the multiplication of antagonistic microorganisms.

2.7.5 Use of resistant cultivars

Cultivated species of potato (*S. tuberosum*) are not resistant to PCN, while related wild potato species and land races are genetically resistant to PCN and resilient to biotic and abiotic stress (Castelli *et al.,* 2003). Currently, over 50 potato species have been tested and identified to be resistant to one pathotype of *Globodera* spp. Most wild potato species originates from Mexico and Andean Highlands and exist in southwestern US and Argentina (Bethke *et al.*, 2017). To identify sources of resistance to PCN in wild potato species, over 1,200 potato cultivars have been screened, leading to the identification of the first resistance gene (H1) to PCN from *S. tuberosum* spp. *andigena*, offering complete resistance to *Globodera rostochiensis* (Gartner *et al.*, 2021).

Potato cultivars Maris Piper and Pentland Javelin from Europe have been used for breeding purposes since they have the H1 resistance gene which is durable to date against PCN (Przetakiewicz and Milczarek, 2017). Moderate resistance cultivars against potato cyst nematodes are Ambition and Acoustin (Gartner *et al*., 2021). On the other hand, potato cultivars Snow gem, Spunta, Trent and Umatilla Australian were susceptible to potato cyst nematodes under greenhouse conditions (Faggian *et al*., 2012). Recently discovered resistant potato cultivars to PCN in Kenya are Caruso, Rhumba and Acaoustin but they are under breeder's rights (Mburu *et al*., 2020).

2.7.6 Bio-controls

From the 1930s to date, the use of natural parasites and biological control agents against potato cyst nematodes has been reported (Saxena. 2018). In the 1990s, most studies were on fungal biocontrol agents; Pochonia, Hirsutella, Arthrobotrys and bacteria Pasteuria against potato cyst nematodes but had challenges in their parasitism efficacy due to climatic and geographical variability (Santos. 2013).

Bio-controls are known to produce leucinotoxins, chitinases, proteases or acetic acid, which have an effect on different stages of these plant-parasitic nematodes (Kiewnick. 2010). The leucinotoxins, chitinases, proteases and acetic acid cause changes in the eggshell layers of plant-parasitic nematodes reducing the hatching of juveniles (Kiewnick. 2010). Al Ajrami *et al*. (2016), demonstrated the reliability of bio-controls to penetrate all sedentary stages (eggs and females) of plant-parasitic nematodes after appressoria formation. For instance, *Purpureocillium lilacinus* strains, among its several modes of action against plant-parasitic nematodes, include infecting the sedentary stage during the nematode life cycle i.e. the egg stage (Kiewnick. 2010).

However, the integration of these fungal bio-controls with other methods of potato cyst nematode control is yet to be explored. For example, under field conditions, *Pochonia chlamydospores* were shown to reduce potato cyst nematode density by 51% (Tobin *et al*., 2008). However, the application of *Verticillium leptobactrum* and *Purpureocillium lilacinum*, reduced potato cyst nematodes by 76 and 73%, respectively under greenhouse (Hajji *et al*., 2017). Elsewhere, *Chaetomium globosum* reduced the *Globodera pallida* population by 76% under Laboratory conditions (Kooliyottil *et al*., 2017).

2.7.7 Organic amendments

Organic amendments improve soil biological, physical and chemical properties and change soil microflora and microfauna resulting in the thriving of several beneficial non-plant parasitic nematodes which feed on bacteria, fungus and suppress the occurrence of plant parasitic nematodes (Park *et al.,* 2011; Kankam *et al.,* 2015). Animal manure during the decomposition of organic matter in the soil produces ammonia which stimulates soil microbial biomass and activities to release biocidal substances on the nematode (Osunlola and Fawole. 2015). Animal manure enhances soil aggregate, restricting nematode movement (Pakeerathan *et al.*, 2009).

Green manure has high nematicidal effects due to the presence of nonacosane-10-ol (C29 alcohol) compounds which suppress plant parasitic nematodes (Naz *et al*., 2015). The efficacy of green manure in suppressing plant parasitic nematodes varies with the type of crop/plant material used as green manure and the species of plant-parasitic nematodes targeted (Oka. 2010). Use of different plant species against plant-parasitic nematodes in thirty-nine plant species used as green manure only 22 of this plant's species belong to botanical families that studies have confirmed to be effective against plant parasitic nematodes (McSorley. 2011).

Plant material from Sudan grass and sorghum (*Sorghum sudanense*), cruciferae (*Raphanus sativus*) and rapeseed (*Brassica napus, B. campestris*) are effective against plant-parasitic nematodes (Oka. 2010; McSorley. 2011). Additionally, solid vermicompost green waste (30% leaves and 70% grass) and vermicompost teas (20% concentration), potentially reduce potato cyst nematodes population under field conditions (Renčo and Kováčik. 2015). In Portugal, green manure from cabbage reduces *Globodera rostochiensis* population under greenhouse conditions by 88% (Fatemy *et al.,* 2016). Unlike synthetic nematicides, organic amendments are environmentally friendly and readily available in most developing countries (Quilty *et al.,* 2011).

CHAPTER THREE

REACTION OF POTATO CULTIVARS TO POTATO CYST NEMATODES UNDER GREENHOUSE CONDITIONS

3.1 Abstract

Several measures have been recommended in the control of potato cyst nematodes (PCN) with resistant potato cultivars being considered the most practical and affordable for smallholder potato farmers in Kenya. However, the level of resistance in locally grown potato varieties is yet to be established. A study was conducted to screen Kenyan potato cultivars against potato cyst nematodes under greenhouse conditions. Eleven potato cultivars namely Shangi, Dutch Robijn, Sherekea, Nyota, Roseline tana, Tigoni, Unica, Asante, Chulu, Kenya Mpya and Arka were screened with Desiree (susceptible variety), Manitou (resistant cultivar) as controls. For each potato cultivar, there were two sets of plants with the first set being inoculated with 50 cysts, while the second batch was nematode-free. The treatments were arranged in a completely randomized design and replicated three times. A scale of 1-9, with 9 indicating the highest level of resistance was used in the assessment of disease severity. Nematode infestation caused reductions in plant height, number of stems and stem diameter, across the cultivars, by up to 50, 60 and 33%, respectively compared to uninoculated control. Root mass reduction across the 11 cultivars ranged from 20 to over 100% compared to uninoculated control. The reproductive index of viable eggs across the 11 cultivars was less than one compared to the control (Desiree). Potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana had a severity score of 1-3(>100-5%) and hence considered to be susceptible to PCN, while cultivars Sherekea and Nyota had a severity score of 4-6 (<25-3%) hence considered partially resistant to PCN. This study

has identified two potato cultivars i.e Sherekea and Nyota with resistance with a severity score of 4-6 (<25-3%) that should be integrated into PCN management in smallholder farms.

3.2 Introduction

It is estimated that potato cyst nematodes (*Globodera* spp) cause a 9% loss in potato yield worldwide (Turner and Subbotin, 2013). In Europe, PCN is the second most economically important pest of potato after late blight, with an estimated economic yield loss of £26 million annually and chemical nematicide use estimated at £20 million annually (Twining *et al.*, 2009; NSP. 2017). It has been established that yield loss associated with PCN varies due to environmental conditions, varieties grown and levels of nematode infestation, with every 20 viable eggs/g of soil causing a loss of 2.75 t/ha in potato yield (EPPO, 2013).

Potato cyst nematodes are classified as a strictly quarantined pest in over 100 countries in the world (EPPO, 2017). PCN status under subtropical and tropical conditions in Africa is yet to be established. Potato cyst nematodes in potato growing areas in Kenya have caused a qualitative and quantitative loss, with infested plants having low root mass development, stunted growth and leaf chlorosis with a potential yield loss of up to 80% when the pest is not controlled (Mwangi *et al.*, 2015; Mburu *et al.*, 2018). In predicting the yield losses, different modeling parameters focusing on, the level of initial PCN population at the beginning of the planting season, type of soil, nematicide use, rotation period and use of susceptible/tolerant cultivars have been used (Trudgill *et al.*, 2014).

Management of PCN through crop rotation is challenging since they are known to survive in the soil for a long time with viable eggs surviving for decades in the absence of the host, hence use of crop rotation must be integrated with other methods to lower PCN population density in the soil (Christoforou *et al.*, 2014). The use of trap cropping has been shown to lower the population density of PCN by inducing premature hatching of new cysts (Mimee *et al.,* 2015). This method requires an adequate understanding of the life cycle of the nematode. Among all the methods used to lower PCN population density, the use of resistant varieties in previous studies has proved to successfully manage plant-parasitic nematodes in various parts of the world (Cook and Starr. 2006). For this reason, tolerant traits of plant varieties are an ideal strategy to reduce yield losses to pests and diseases (Peng and Moens. 2002; Cook and Starr. 2006).

Plant resistance to plant-parasitic nematodes is an important aspect in the management of PCN given the concerns over environmental contamination caused by the continuous use of chemical pesticides (Peng and Moens. 2002). Over 1,200 wild potato species have been screened for resistance to PCN and the first PCN resistance gene (*H1*) from *S. tuberosum* spp. *andigena*, offering complete resistance to *Globodera rostochiensis* pathotypes Ro1 and Ro4 in potatoes has been identified (Whitworth *et al.*, 2018).

Cultivated species of *Solanum tuberosum* do not show resistance to PCN, however, wild relatives and landraces have been tested and shown to be genetically resistant to potato cyst nematodes and abiotic stress (Castelli *et al.*, 2003). Currently, over 50 potato species have shown some resistance to one pathotype of *Globodera* spp. Mexico, the US, and Argentina are reported to have these wild potato species in their natural habitat (Bethke *et al.*, 2017). This study was conducted to determine the reaction of potato cultivars to potato cyst nematodes under greenhouse conditions in Kenya.

3.3 Materials and methods

3.3.1 Experimental site and cultivar selection

A greenhouse experiment was conducted from March to August 2021 at the University of Nairobi-Upper Kabete campus (1° 15'S and 36° 44'E), with an elevation of 1,820 m above sea level. Eleven potato cultivars commonly grown by Kenyan farmers; Dutch Robijn, Shangi, Roslin Tana, Tigoni, Asante, Sherekea, Nyota, Kenya Mpya, Unica, Chulu and Arka were selected for the study. Potato cultivars Desiree (susceptible) and Manitou (resistant) potato cultivars were included as controls. Certified potato tubers were obtained from the Kenya Agricultural and Livestock Research Organization – Tigoni.

3.3.2 Preparation of potato cyst nematodes inoculum

Potato cyst nematodes inoculum was extracted from naturally infested fields in Nyandarua County. The Fenwick Can method (Fenwick, 1940), was used to extract PCN cysts from infested soil. The viability of the eggs within the cysts was verified by soaking a random sample of 10 cysts per soil sample in 0.001% Nile blue stain (*v/v*) for 48hr (Faggian *et al.,* 2012). Dead eggs or juveniles stained blue while the live ones did not stain after opening the cyst. Samples with at least 50% of either live eggs or juveniles were used as the sources of inoculum. Each Eppendorf tube was used to preserve 50 cysts until needed.

3.3.3 Set up of greenhouse experiment

Eleven chitted potato cultivars were sown in plastic pots containing 400g of soil and sand (steam-sterilized at 180° C for 30 minutes) mixed at a ratio of 3:1 (*v/v*). Two weeks later, 50 cysts of PCN having about 100 eggs/ cyst were introduced into each pot as described by Whitworth *et al.* (2018). Each potato cultivar was in two batches after sowing in three pots and replicated three times. Fifty cysts of the nematodes were introduced into each pot, while the other nine pots were nematode-free (non-inoculated) and treatments were arranged in a completely randomized design. Plants were watered at intervals of four days.

3.4 Data collection

Data on plant height, stem diameter, number of stems, root mass, cyst count and egg viability from each potato cultivar were collected 70 days after planting. Final population (P_f) densities of cyst and egg viability per 3 cysts of each treatment were evaluated, following cyst extraction, picking and counting under a dissecting microscope at 40x magnification.

3.5 Data analysis

Data on plant growth (height, number of stems, stem diameter, root mass) were compared on a *t*-test whereby a p-value less than 5% was considered significantly different. Potato cyst nematodes and egg reproduction index was expressed as RI = Pf /Pi (Van Den Berg and Rossing 2005). The susceptibility rating of the eleven potato cultivars to PCN was determined by calculating, PF (final cyst population of the test variety)/ PF (final cyst population of standard susceptible control variety i.e. Desiree) × 100, while the severity score was divided into 9 susceptibility groups i.e. 1 (>100), 2 (50.1–100%), 3 (25.1–50%), 4 (15.1–25%), 5 (10.1–15%), 6 (5.1–10%), 7 (3.1–5%), 8 (1.1–3%), 9 (≤1%), with 1-3 being susceptible, 4-6 partially resistant and 7-9 resistant as per EPPO protocol (2006). The data on cyst and egg counts were normalized using square root transformation before analysis of variance (Gomez, 1984). Thereafter, an analysis of variance using GenStat (15^{th} edition) statistical software was done and means of cysts and egg counts in potato cultivars were compared and separated using Tukey's least significant difference test (p<0.05).

3.6 Results

3.6.1 Effects of potato cyst nematodes infections on the growth of different potato cultivars

There was a significant difference ($p \leq 0.05$) in plant height, stem number and stem diameter of nematode-infected plants compared to non-inoculated controls (Tables 3.1, 3.2 and 3.3). Potato cyst nematode infection caused a 40 and 38 % reduction in the number of stems in potato cultivars Desiree (susceptible cultivar) and Manitou (resistant cultivar), respectively. Significant reduction in height was in potato cultivars Nyota and Chulu at 29%, followed by Arka at 25% and was comparable to Desiree (susceptible cultivar). Potato cultivars Unica and Kenya Mpya had the least reduction in plant height by 10%. The rest of the potato cultivars reduced in plant height by an average of between 10 and 20%. The number of stems was significantly reduced in cultivars Tigoni and Unica by 51 and 28% respectively. The reduction in the number of stems was higher than 20% in other cultivars (Table 3.2).

Non-inoculated controls had slightly wider stem girths of 5.1 mm than those infested with PCN. Cultivars Arka, Roseline tana and Unica had a ≤ 10% reduction in stem girth and were comparable to Manito (resistant cultivar) which had a 4% reduction in stem girth. The highest reduction in stem girth was in cultivars Dutch Robijn 27%, followed by Asante (23%) and Kenya Mpya (21%). Cultivars Nyota and Tigoni had a 16% reduction in stem girth followed by Sherekea 12%, and Shangi 11% and were comparable to Desiree (susceptible cultivar) which had a 14% reduction in stem girth (Table 3.3).

Table 3. 1: Effect of potato cyst nematodes infestation on plant height of different potato cultivars

	Season one				Season two			
Potato cultivars	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in height	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in height
Shangi	65.5a	84.6a	0.2	29	38.5a	42.3a	0.7	10
Dutch Robijn	69.8a	78.2a	0.5	12	29.1a	34.7a	0.4	19
Sherekea	53.6a	68.1a	1.7	27	35.4a	39.9a	0.5	13
Desiree	61.4a	72.7a	0.4	18	17.5a	25.0a	0.3	43
Nyota	55.3a	83.0a	0.2	50	31.9a	34.4a	0.7	8
Roseline Tana	65.8a	76.9a	0.5	17	24.4a	27.5a	0.7	13
Tigoni	79.2a	96.2a	0.1	21	50.5a	57.9a	0.5	15
Unica	69.9a	76.7a	0.5	9	31.3a	34.6a	0.7	10
Asante	76.2a	82.4a	0.7	8	32.6a	43.3a	0.1	33
Chulu	58.9a	85.9a	0.4	46	29.0a	32.5a	0.5	12
Kenya Mpya	64.3a	70.6a	0.6	10	41.3a	45.5a	0.3	9
Arka	35.7a	51.6a	0.3	45	27.1a	28.2a	0.8	4
Manitou	63.9a	71.2a	0.5	11	31.4a	41.6a	0.1	33
S.E.M	18.9	13.6			5.95	7.4		
L. S. D	31.7	22.9			17.3	21.5		
CV%	22.2	13.3			23.5	26.1		

Means of plant height in inoculated and non-inoculated plants across the eleven potato cultivars were based on a t-test with $p< 0.05$. Means of plant height of either inoculated or non-inoculated with the same letters are not significantly different at ($p \leq 0.05$). CV(%) =Coefficient of Variation, LSD=Least Significant Difference, S.E.M=standard error of means.

Table 3.2: Effect of potato cyst nematodes infestation on stem number of different potato cultivars

	Season one				Season two			
Potato cultivars	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in stem number	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in stem number
Shangi	3.1a	3.6a	0.3	18	3.4a	4.3a	0.5	24
Dutch Robijn	7.3a	8.0a	0.5	10	8.1a	8.5a	0.9	5
Sherekea	4.1a	4.7a	0.4	16	8.3a	9.2a	0.4	10
Desiree	2.1a	3.0a	0.5	41	1.8a	2.4a	0.6	34
Nyota	4.2a	5.1a	0.5	21	7.4a	8.2a	0.7	11
Roseline Tana	3.0a	3.9a	0.3	32	3.3a	3.3a	0.8	0.6
Tigoni	4.3a	6.1a	0.3	42	5.2a	8.3a	0.1	60
Unica	4.4a	5.5a	0.5	26	2.4a	3.1a	0.5	29
Asante	4.3a	5.0a	0.6	17	6.4a	6.4a	0.7	0.5
Chulu	3.0a	3.3a	0.6	12	2.9a	3.6a	0.5	25
Kenya Mpya	2.8a	3.7a	0.3	32	4.1a	4.6a	0.5	12
Arka	3.2a	4.4a	0.3	40	5.9a	6.1a	0.9	3
Manitou	2.7a	3.6a	0.4	30	3.5a	5.3a	0.2	53
S.E.M	3	3.2			3.2	3.5		
L. S. D	4.7	5.4			5.3	5.9		
CV%	55.6	50.9			49.2	45.8		

Means number of stem in inoculated and non-inoculated plants across the eleven potato cultivars were based on a t-test with $p < 0.05$. Means of number of stems of either inoculated or non-inoculated with the same letters are not significantly different at ($p \leq 0.05$). CV(%) =Coefficient of Variation, LSD=Least Significant Difference, S.E.M=standard error of means.

Table 3.3: Effect of potato cyst nematodes infestation on stem diameter of different potato cultivars

	Season one				Season two			
Potato cultivars	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in stem diameter	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in stem diameter
Shangi	5.0a	5.5a	0.6	10	4.1a	5.0a	0.5	21
Dutch Robijn	3.9a	5.2a	0.2	33	3.5a	4.2a	0.4	20
Sherekea	3.9a	4.3a	0.6	22	4.1a	4.2a	0.3	2
Desiree	3.7a	4.2a	0.6	12	3.0a	3.4a	0.7	15
Nyota	3.9a	4.2a	0.5	10	2.6a	3.1a	0.3	22
Roseline Tana	3.9a	4.6a	0.5	16	3.0a	3.3a	0.8	9
Tigoni	4.4a	5.5a	0.4	24	5.8a	6.3a	0.6	8
Unica	3.5a	4.0a	0.6	16	3.8a	3.9a	0.9	3
Asante	3.9a	4.8a	0.4	24	4.0a	4.9a	0.3	21
Chulu	4.7a	6.4a	0.3	36	5.9a	6.7a	0.5	13
Kenya Mpya	4.3a	5.3a	0.4	21	6.8a	8.2a	0.2	20
Arka	3.8a	4.2a	0.6	10	3.2a	3.4a	0.8	8
Manitou	4.2a	4.2a	0.5	1	3.7a	3.9a	0.7	7
S.E.M	2.1	2.6			2.6	3.0		
L. S. D	3.5	4.4			4.4	5.0		
CV%	38.6	41.4			47.8	47.8		

Means of stem diameter in inoculated and non-inoculated plants across the eleven potato cultivars were based on a t-test with $p < 0.05$. Means of stem diameter of either inoculated or non-inoculated with the same letters are not significantly different at ($p \leq 0.05$). CV(%) =Coefficient of Variation, LSD=Least Significant Difference, S.E.M=standard error of means.

3.6.2 Effect of potato cyst nematodes infections on root mass of different potato cultivars

Potato cyst nematode infestation caused a decrease in root mass of the 11 potato cultivars compared to their controls 70 days after planting. There was a significant difference ($p \leq 0.05$) in the root mass of nematode-infected plants compared to non-inoculated controls (Table 4). Cultivar Arka had significantly reduced root mass between inoculated and non-inoculated treatment and was comparable to Desiree (susceptible cultivar). Amongst the 11 cultivars tested, Arka, Tigoni and Unica had a significant reduction in root mass by >100%. Cultivars Sherekea and Chulu had the least reduced root mass by 2.5 and 3.3 % respectively. Root mass reduction ranged from 20% to 75% in other potato cultivars (Table 3.4).

Table 3.4: Effect of potato cyst nematodes infestation on root mass of different potato cultivars

	Season one				Season two			
Potato cultivars	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in root mass	Inoculated	Non-inoculated	P-value between inoculated and non-inoculated potato cultivars	% Decrease in root mass
Shangi	6.5abc	10.6ab	0.1	62	38.2ab	67.1abc	0.9	75
Dutch Robijn	7.2bc	13.4ab	0.2	87	51.0bc	80.4bc	0.3	58
Sherekea	4.6ab	4.8ab	0.9	2	31.6ab	32.7ab	0.9	3
Desiree	2.7ab	4.1a	0.05*	48	19.5ab	32.4ab	0.5	66
Nyota	3.6ab	4.6a	0.4	28	20.4ab	23.1ab	0.6	14
Roseline Tana	4.2ab	5.4ab	0.14	28	20.9ab	23.9ab	0.3	14
Tigoni	5.2ab	12.0ab	0.06	132	16.6ab	37.4ab	0.09	125
Unica	6.7abc	14.2b	0.16	111	11.8ab	22.9a	0.8	93
Asante	3.7ab	6.3ab	0.24	71	24.4ab	39.1ab	0.1	60
Chulu	10.5c	11.1ab	0.82	6	39.7abc	40.0ab	0.2	0.6
Kenya Mpya	3.4ab	4.5a	0.08	34	83.2c	98.0c	0.5	18
Arka	1.7a	4.1a	0.04*	142	6.1a	17.2a	0.7	183
Manitou	4.1ab	5.6ab	0.3	35	21.9ab	25.7ab	0.6	17
S.E.M	2.9	5.3			25	32.2		
L. S. D	5.1	9.5			44.5	57.3		
CV%	72.4	53.8			32.9	16.2		

Means of root mass in inoculated and non-inoculated plants across the eleven potato cultivars were based on a t-test with $p< 0.05$. Means of number of root mass either inoculated or non-inoculated with the same letters are not significantly different at ($p \leq 0.05$). CV(%) =Coefficient of Variation, LSD=Least Significant Difference, S.E.M=standard error of means.

3.6.3 Potato cyst nematodes population density on different potato cultivars

The final PCN population density was significantly different ($p \leq 0.05$) among the cultivars in both seasons one and two. Cultivars Manitou(resistant cultivar) and Nyota had significantly reduced final cyst population by 14 -67% compared to control, while cultivars Sherekea, Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana had increased final cyst population of up to 300% compared to the control. Cultivars Nyota and Manitou had the lowest reproductive index of RI=0.6 and RI=0.53, followed by Sherekea with the reproductive index of RI=1.18 compared to Desiree control. Cultivars (Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya, Roseline Tana) had a reproductive index of >1(Table 3.5). The severity score of cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana was 1-3 hence considered susceptible to potato cyst nematodes, while Sherekea and Nyota had a severity score of 4-6 hence considered partially resistant to potato cyst nematodes (Table 3.6).

Table 3.5: Cyst numbers and reproductive index (RI) of potato cyst nematodes in different potato cultivars

Potato cultivars	Season one			Season two		
	Final cyst count(Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population(Pi)	Final cyst count(Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population(Pi)
Desiree	241.0 e	4.82 e	382	170.7 h	3.41 h	241
Shangi	172.7 cde	3.45 cde	245	122.3 g	2.45 g	145
Tigoni	200.0 de	4.00 de	300	98.0 f	1.96 f	96
Dutch Robijn	158.3 bcd	3.17 bcd	216	87.7 f	1.75 f	75
Chulu	151.7 bcd	3.03 bcd	203	85.0 ef	1.70 ef	70
Asante	109.0 abc	2.18 abc	118	93.0 f	1.86 f	86
Unica	95.3 abc	1.91 abc	91	70.7 de	1.41 de	41
Arka	107.7 abc	2.15 abc	115	66.0 cd	1.32 cd	32
Kenya Mpya	95.3 abc	1.91 abc	91	64.0 cd	1.28 cd	28
Roseline Tana	69.0 ab	1.38 ab	38	53.3 bc	1.07 bc	6
Manitou	34.7 a	0.69 a	-31	18.7 a	0.37 a	-63
Sherekea	70.7 ab	1.41 ab	41	47.0 b	0.94 b	-6
Nyota	43.0 a	0.86 a	-14	16.3 a	0.33 a	-67
S.E.M	34.5	0.69		18.0	0.36	
L. S. D	100.2	2.00		15.3	0.31	
CV(%)	50.1	50.1		40.8	40.8	

Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV (%) =Coefficient of Variation, LSD=Least Significant Difference, Initial Population (Pi) = 50 cysts, reproductive index (RI=Pf/Pi).

Table 3.6: Susceptibility rating of potato cultivars to potato cyst nematodes

Potato cultivars	Season one		Season two	
	Severity score	% Susceptibility score	Severity score	% Susceptibility score
Desiree	2	100	2	100
Shangi	2	72	2	72
Tigoni	2	57	2	83
Dutch Robijn	2	51	2	66
Chulu	2	50	2	63
Asante	2	54	3	45
Unica	3	41	3	40
Arka	3	39	3	45
Kenya Mpya	3	37	3	40
Roseline Tana	3	31	3	29
Manito	4	11	4	14
Sherekea	4	25	4	24
Nyota	4	10	4	18

Severity score; 1-3 susceptible, 4-6 partially resistant and 7-9 resistant.

3.6.4 Reproductive index of potato cyst nematodes viable eggs on different potato cultivars

There was a significant difference ($p \leq 0.05$) in viable eggs across the varieties tested in seasons one and two. All the 11 cultivars had a reproductive index of less than 1 compared to Desiree which was susceptible control (Table 3.7)

Table 3.7: Reproductive index of potato cyst nematodes viable eggs on different potato cultivars

Potato cultivars	Season one		Season two	
	Final egg count (Pf)/cyst	RI (Pf/Pi)	Final egg count (Pf)/cyst	RI (Pf/Pi)
Chulu	35.0 a	0.35 a	55.0 abc	0.55 abc
Shangi	90.7 ab	0.9 ab	98.7 cd	0.99 cd
Unica	68.7 ab	0.69 ab	74.0 abc	0.74 abc
Nyota	24.7 a	0.25 a	89.0 bcd	0.89 bcd
Dutch Robijn	30.3 a	0.30 a	34.3 ab	0.34 ab
Kenya Mpya	64.7 ab	0.65 ab	89.0 bcd	0.89 bcd
Tigoni	48.3 ab	0.48 ab	28.7 a	0.29 a
Sherekea	32.3 a	0.32 a	84.3 abcd	0.84 abcd
Roseline Tana	30.0 a	0.30 a	48.3 abc	0.48 abc
Manitou	52.3 ab	0.52 ab	67.7 abc	0.68 abc
Desiree	114.3 b	1.14 b	139.7 d	1.4 d
Arka	70.0 ab	0.70 ab	84.7 abcd	0.85 abcd
Asante	84.7 ab	0.85 ab	69.7 abc	0.7 abc
S.E.M	24.38	0.24	19.51	2.0
L. S. D	70.89	0.71	56.71	0.57
CV(%)	63.9	63.9	45.6	45.6

Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%) =Coefficient of Variation, LSD=Least Significant Difference, Reproductive index (RI) = Pf/Pi, Pi- initial population (100 viable eggs).

3.7 Discussion

Findings in this study showed that inoculation of different potato cultivars with PCN had no significant effect on plant height, stem number and girth. Amongst the eleven cultivars, cultivars Nyota, Sherekea, Tigoni, Shangi, Chulu and Arka were significantly stunted and necrotic, while cultivars Unica, Kenya Mpya and Roseline tana were taller. Stunting and necrosis are an indications that PCN interferes with plant growth as a result of piercing the cells, creating feeding tubes as they pass through the root systems of potato cultivars Nyota, Sherekea, Tigoni, Shangi, Chulu and Arka making these potato cultivars stressed with premature senescence. Cultivars Unica, Kenya Mpya and Rosline tana degraded syncytia and cytoplasm preventing the nematode from molting to the adult stage due to the presence of H3 resistance gene (Grunewald *et al*., 2009; Varypatakis *et al.*, 2020). These findings are comparable to those obtained by Urek *et al*. (2008); Hajji-Hedfi. (2017); Mezerket *et al.* (2018), reported that resistant potato cultivar Kudor was able to grow taller with thick stems in PCN-infested fields, while susceptible potato cultivars Spunta and Desiree were stunted and necrotic.

Potato cyst nematodes had a significant effect on the root mass of different potato cultivars during their growth. Potato cultivar Arka had significantly lower root mass followed by, Tigoni and Unica among the eleven potato cultivars. PCN J2 penetrated and developed in the root systems of potato cultivars Arka, Tigoni and Unica forming feeding tubes that limited the absorption of water and nutrients (Urek *et al.* 2008; Sudha *et al.* 2017). Cultivars Chulu and Sherekea had higher root density due to the ability to reproduce extra roots when infested with PCN hence increasing their efficiency in nutrient and water uptake (Gartner *et al.*, 2021). These findings are reflected by Thorpe *et al.* (2014) and Mei *et al*. (2015), who reported that host-nematode defense response to resistance and susceptible potato cultivars in the root system at the early stage are affected by PCN effectors, which are important in syncytia formation, hence suppressing defense response in susceptible cultivars, unlike resistant cultivars.

There was a significant difference in final PCN population density among the cultivars in seasons one and two. Cultivars Nyota and Sherekea significantly lowered the final cyst population density and reproductive index amongst the eleven potato cultivars tested, while the rest of the potato cultivars increased the final cyst population by up to 300%. The reproductive index of encysted viable eggs was below one across the eleven potato cultivars compared to the susceptible cultivar (Desiree). The difference in the final cyst population on susceptible potato cultivars is dependent on different PCN species and pathotypes, as the same pathotypes have different population densities across different susceptible cultivars (Sudha *et al.*, 2016). On the other hand, the increase in the number of cysts and viable eggs in susceptible potato varieties was because of their sensitivity toward PCN (Urek *et al.*, 2008; Hajji-Hedfi. 2017; Mezerket *et al.*, 2018). A decrease in the number of cyst and viable eggs in resistant potato cultivars Nyota and Sherekea was due to the presence of H1 resistance gene which prevents the nematode from molting to the adult stage (Simko *et al.*,. 2007).

The severity score of potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana showed that they were susceptible to PCN, while potato cultivars Sherekea and Nyota were partially resistant/tolerant to potato cyst nematodes. Resistance of potato cultivars to *G. rostochiensis* is attributed to several genes, which confer partial or complete resistance (Finkers-Tomczak *et al.*, 2011). The H1 gene confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis,* which inhibits the multiplication of PCN juveniles to develop into females (Simko *et al.,* 2007, Finkers-Tomczak *et al.*, 2011). These findings are in concordant with Sudha *et al.* (2016), who showed that cultivar Kufri Swarna was resistant to PCN pathotype Ro1, 4, Pa2, 3 Pa2/3 but susceptible to pathotype Ro5. Reproduction levels of plant parasitic nematodes on plant tissues are used to measure resistance while damage levels are used to quantify tolerance (Bethke *et al.*, 2017).

CHAPTER FOUR

EFFICACY OF ORGANIC AMENDMENTS IN THE CONTROL OF POTATO CYST NEMATODES UNDER GREENHOUSE AND FIELD CONDITIONS

4.1 Abstract

Potato cyst nematodes are invasive, fast-spreading and well-adapted pests of potatoes. Establishment and spread of PCN have the potential of causing severe losses in potato production, hence a threat to food and nutritional security. This study was set to determine the efficacy of organic amendments in the control of PCN under greenhouse and field conditions. Animal and green manures were applied into sterilized sandy-loam soil 2:1 (*w/w*), in 4 kg plastic pots, at the rate of (20 t/ha), with the commercial neem-based product registered for controlling root-knot nematodes being included as a control. Chitted potato cultivar Shangi tubers were sown and 50 PCN cysts were introduced into each pot. Treatments were arranged in a completely randomized design with six replications under the greenhouse and a split-plot design with potato varieties as the main plot and organic amendments as subplots under field. Data collected was on plant growth parameters, cyst count and viable eggs. Under greenhouse, pots amended with organic manure increased plant height and stem girth by up to 29 and 48% respectively, except in chicken manure-amended soil where plant height and stem girth was reduced by 20 and 14% respectively compared to the control. Organic manure reduced cyst count by 50%. Plants grown in soil amended with pig, cow and goat manures had up to three times increased root mass, while plants grown in soil amended with green and chicken manure had lower root mass compared to unamended control. Under field, the organic amendments caused an increase in plant height and stem girth by up to 36 and 25% compared to the control. Animal (cow, goat) and green manures increased in root mass

by 52 and 23% respectively. The final cyst population density was reduced by 85% with a reproductive index of <1 in all treatments compared to control. Organic amendments increased total yield by 53% across the varieties. This study has demonstrated that organic amendments have enormous potential in the control of PCN and the improvement of plant growth.

4.2 Introduction

Potato (*Solanum tuberosum*) is the second most important staple food crop after maize (CIP, 2019), with over 2.5 million people benefiting from the whole potato value chain in Kenya (Abong and Kabira. 2013). On the global scale, about half of potato production comes from Asia, especially China, while Europe follows with approximately a third (Scott and Suarez, 2012). Africa accounts for around 7% or so of the globally produced potatoes led by Egypt and South Africa (Devaux *et al*., 2014). Kenya has the potential of producing potatoes at 20-40 t/ha, but the substandard seed quality, inappropriate cropping practices, pests and diseases have led to low productivity of about 9-10 t/ha (Kiptoo *et al*., 2016; VIB. 2019).

Potato cyst nematodes are considered one of the most important pests of potatoes around the globe (Coyne *et al*., 2018; Niere and Karuri, 2018). The nematode was first detected in the southern Peruvian Andes and has since spread throughout the world (Grenier *et al.*, 2010; Alonso *et al*., 2011; Moens *et al*., 2018: EPPO, 2020). There have been reports of potato cyst nematodes in potato-growing regions of Europe, America, Asia, Oceania and Africa (EPPO, 2020). In Kenya, PCN was initially discovered in 2015 and has since spread to all the main potato-growing regions, posing a danger to potato output and food security. (Mwangi *et al*., 2015; Mburu *et al.*, 2018). Potato cyst nematode has posed a challenge in terms of the cost incurred to manage the pest by small-scale farmers who are dependent majorly on potato for their livelihoods (Mburu *et al.*, 2020).

Management strategies comprising resistant potato cultivars, crop rotation, use of bio-controls and non-fumigant nematicides, are promising in reducing potato cyst nematodes population density in the soil (Desgarennes *et al.,* 2018). However, the persistence of the nematodes in the soil for up to two decades in absence of an alternate host and prolonged desiccation period limits the efficacy of some of the strategies (Christoforou *et al.,* 2014). Thus, additional, environmentally friendly strategies are being promoted as research on new and sustainable management strategies continues (Verginer *et al.,* 2010). Organic amendments improve soil fertility, reduce plant parasitic nematode density and enhance crop production across different farming systems (Takahiro *et al.,* 2015; Li *et al.,* 2018; Luo *et al.,* 2018; El-Ashry, 2021).

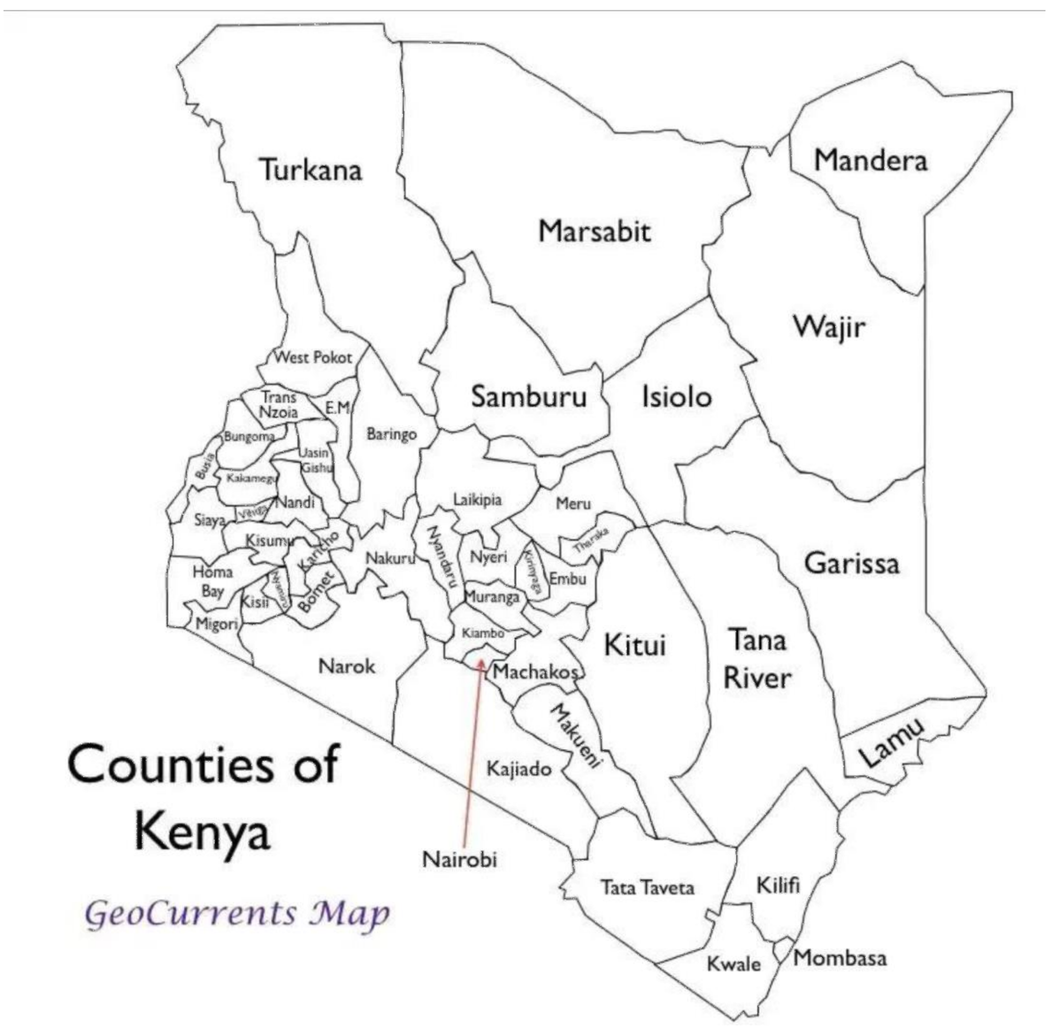
Various studies have revealed that organic manures from animals and plants effectively reduced lesion nematodes (*Pratylenchus* spp), root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp) population densities (Min *et al.,* 2007; Liang *et al.,* 2009). This suppression is achieved when organic matter breaks down to release toxic ammonia, which increases natural nematode-antagonist microorganisms in the soil, as well as inducing systemic plant resistance (Thoden *et al.,* 2011). There is insufficient information concerning the management of PCN using organic amendments in Kenya. Therefore, this research was conducted to determine the efficacy of animal and green manures in the control of potato cyst nematodes under greenhouse and field conditions in Kenya.

4.3 Materials and methods

4.3.1 Experimental sites

Greenhouse experiments were conducted from March to May and June to August 2021 at the University of Nairobi- upper Kabete field station situated (10 15′S, 360 44′E) at an altitude of 1,820m (Sambroek *et al*., 1982). Field experiments were conducted during long and short rainy seasons from April to December 2021 at Nyandarua County-Murungaru sub-county situated (0.5676’S and 36.4689’E) at an altitude of 2384 m, with an annual rainfall of between 700-1500 mm (Figure 4.1).

Figure 4.1: Geographical location of Nyandarua County, Kenya



4.3.2 Organic amendment and potato tuber selection

Organic amendments widely used by local farmers namely: animal manure (cow, pig, goat and chicken) and green manure (cabbage leaves) for use under greenhouse experiments were sourced from University of Nairobi- Kanyariri Farm, while animal manure (cow and goat) and green manure for use under field experiment were sourced from neighboring farms in Murungaru sub-county. Certified seeds of potato cultivars Desiree (susceptible to PCN), Shangi (moderately tolerant to PCN) and Markies (tolerant to PCN) that are commonly grown by local farmers were obtained from the Kenya Agricultural and Livestock Research Organization-Tigoni.

4.3.3 Preparation of potato cyst nematodes inoculum

PCN inoculum used for the greenhouse experiment was obtained from naturally infested fields in Nyandarua County. The Fenwick Can method (Fenwick, 1940), was used to extract PCN cysts from infested soil. The viability of the eggs within the cysts was verified by soaking a random sample of 10 cysts per soil sample in 0.001% Nile blue stain (*v/v*) for 48hr (Faggian *et al.,* 2012). Dead eggs or juveniles stained blue while the live ones did not stain after opening the cyst. Samples with at least 50% of either live eggs or juveniles were used as the sources of inoculum. The cysts were extracted and placed in eppendorf tubes with each tube being used to preserve 50 cysts until needed.

4.3.4 Set up of greenhouse experiment

Sandy-loam soil sterilized for 30 min at 180^0C in an oven was mixed at 2:1 (*w/w*) in a 4kg plastic pot. Animal manure (cow, pig, goat and chicken), green manure (cabbage leaves chopped into small pieces) were incorporated into sterile sandy-loam soil at the rate of 20 t/ha (Thoden *et al.* 2011) and 45 t/ha wet weight respectively (Valdes *et al.,* 2011; Fatemy and Sepideh, 2016), the C: N ratio was determined by the dry combustion method using a CN analyzer (Table 4.1). The negative control was amendment-free sandy-loam soil, while the positive control was sandy–loam soil incorporated with neem extract (6L/ha) which has been registered to manage root-knot nematodes. Chitted Shangi potato variety was sown in each pot. Plants were inoculated with 50 cysts (Whitworth *et al*., 2018), 14 days after planting. The experiment was designed in a completely randomized design (CRD), each treatment had four pots replicated six times and plants were watered after four days. At the end of the experiments, all soil was drenched with 10% (*v/v*) of sodium hypochlorite and allowed to settle for about five days before autoclaving for at least four hours.

4.3.5 Set up of field experiment

The field trial was conducted on a naturally infested farm with Potato cyst nematodes in a split-plot design replicated three times, with the main plot being varieties Desiree, Shangi and Markies, while subplots (cow manure, goat manure and green manure which significantly reduced PCN population densities under greenhouse conditions). Cow and goat manure was applied at the rate of 20 t/ha dry weight and green manure was chopped and mixed into the soil at the rate of 45 t/ha wet weight at planting. Negative control was unamended plot while positive control was plots applied with commercial neem extract at the rate of (6 L/ha). The experimental unit measured 9 m^2 with a spacing of 75 cm by 30 cm. Soil samples (200 cm^3) from each plot were collected before planting, 45 days after planting and at harvest to determine cyst/200 cm^3 and egg counts/cyst. The crops were rain-fed and kept free from weeds, pests and diseases through good agronomic practices and chemical spraying during the growing season.

4.3.6 Extraction of potato cyst nematodes

Each soil sample from Nyandarua County was homogenized and a sample of 200 cm^3 of potato cyst nematode-infested soil was taken and the cysts were extracted using the Fenwick Can method (Fenwick, 1940).

4.4 Data collection

Data on plant growth parameters, final cyst count in 200 cm^3 of soil from each pot and viability test per 3 cysts per pot were recorded 70 days after planting under greenhouse conditions. Field data on plant growth parameters were determined 90 days after planting, while data on tuber yield per plot were recorded at harvest. Total yield was further split into ware (>55 mm), seeds (25-55 mm) and chats (<25 mm) based on tuber diameter. Initial (Pi) and final (Pf) cyst population densities in 200 cm^3 of soil and egg viability per 3 cyst/treatments were determined at planting and harvesting respectively.

4.5 Data analysis

Data on cyst and egg counts were normalized using square root transformation before analysis of variance (Gomez, 1984). Potato cyst nematodes and viable eggs' reproductive index was expressed as RI = Pf /Pi (Van Den Berg and Rossing 2005). All the collected data on plant parameters, cyst counts and viable eggs were subjected to ANOVA test with Tukey multiple comparisons to determine statistical significance using GenStat software (15th Edition). Treatment means were separated using least significant differences (LSD) tests at a 5% level of significance.

4.6 Results

4.6.1 Chemical properties of organic amendments

Animal manure (cow, goat and pig) had moderately narrow C:N ratio, while chicken manure had narrower C:N ratio as green manure recorded higher C:N ratio (Table 4.1).

Table 4.1: Chemical properties of organic amendments used for amending soil in greenhouse and field trials

Organic amendment	C (g/kg)	N (g/kg)	C:N
Goat manure	21.4	1.08	19.81
Cow manure	23.9	1.21	19.75
Chicken manure	28.7	2.14	13.41
Pig manure	17.1	0.92	18.59
Green manure	40.2	1.85	21.73

4.6.2 Greenhouse experiment

4.6.2.1 Effects of organic amendments on the growth of Shangi potato cultivar inoculated with potato cyst nematodes

Application of organic amendments, except for chicken manure caused a significant increase in plant growth compared to unamended control. Overall, there was a significant difference ($p<0.05$) in the growth of potato crops in both seasons after organic amendment application. Plants inoculated with PCN in pots amended with pig manure were taller by 29%, followed by goat manure (25.5%), cow manure (22%) and green manure (20.5%) compared to unamended control. Inoculated plants amended with chicken manure were shorter by 14% compared to the unamended control (Table 4.2). The average number of stems during plant growth period ranged between 3 to 5 stems per crop across the treatments compared to unamended control. Stem diameter during plant growth increased by 4.8-8.8 mm in inoculated plants amended with cow manure, followed by pig manure (5.7-7.2 mm), green manure (4.8-5.9 mm) and goat manure (4.5-5.6 mm) and did not differ statistically from the unamended inoculated plant. Inoculated plants amended with chicken manure had thin stems (2.9-5.0 mm) which were significantly different from unamended inoculated plants (Table 4.2).

Table 4.2: Effects of organic amendments on the growth of potato cultivar Shangi in soil infested with potato cyst nematodes

Organic Amendments	Season one				Season two			
	Plant height (cm)	Stem Number	Stem girth (mm)	%change in height over control	Plant height (cm)	Stems Number	Stem girth (mm)	%change in height over control
Control	57.5 b	1.0 a	4.6 b		85.3 ab	3.8 bc	5.3 ab	
Neem extract	53.9 ab	1.2 a	4.6 b		81.6 ab	4.2 c	5.0 ab	
Green manure	84.0 c	1.8 a	5.9 b	46	81.2 a	3.4 abc	4.8 a	-5
Chicken manure	49.8 a	1.7 a	2.9 a	-13	72.0 a	3.0 ab	5.0 ab	-16
Pig manure	83.2 c	3.6 b	5.7 b	45	96.5 b	2.8 ab	7.2 ab	13
Cow manure	83.9 c	4.4 bc	4.8 b	46	83.5 ab	2.7 a	8.8 b	-2
Goat manure	85.7 c	5.4 c	4.5 b	49	87.0 ab	3.1 ab	5.6 ab	2
S.E.M	21.3	0.4	0.6		5.4	0.4	1.4	
L. S. D	7.6	1.1	1.6		15.1	1.0	3.8	
C.V(%)	18.3	3.7	2.5		26.5	10.1	6.7	

Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means.

4.6.2.2 Effects of organic amendments on root-mass of potato cultivar Shangi inoculated with potato cyst nematodes

Inoculated plants amended with animal and green manure with exception of chicken manure significantly increased potato root mass compared to unamended control. Overall, there was a significant difference ($p<0.05$) in the root mass of potato crop in both seasons one and two after organic amendment application. Inoculated plants amended with pig manure, goat and cow manure significantly increased in root mass by ≥ 100% and were significantly different from inoculated unamended control. Inoculated plants amended with chicken manure and green manure had a 9 and 14% reduction in root mass respectively and did not differ significantly from the unamended control (Table 4.3).

Table 4.3: Effects of organic amendments on root mass of potato cultivar Shangi inoculated with potato cyst nematodes

	Season one		Season two	
Organic amendment	Root mass (g)	%change in rootmass over control	Root mass (g)	%change in rootmass over control
Control	15.6 a		27.7 ab	
Neem extract	19.0 a		29.3 abc	
Green manure	16.8 a	7	21.8 a	-21
Chicken manure	11.8 a	-24	31.8 abc	15
Pig manure	38.8 b	148	42.0 cd	52
Cow manure	48.5 bc	210	51.0 d	84
Goat manure	54.2 c	246	37.3 bcd	35
S.E.M	4.7		4.9	
L. S. D	13.1		13.7	
CV (%)	30.1		24.1	

Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means.

4.6.2.3 Effects of organic amendments on potato cyst nematodes population density

Organic amendments had significantly reduced PCN population density on potato cultivar Shangi compared to inoculated unamended control (Table 4.4). Treatments with cow manure reduced final cyst population density by 80%, followed by goat manure (79%), green manure (72%), pig manure (67%) and chicken manure (64%), compared to inoculated unamended control. Goat manure had the lowest cyst reproductive index of RI=0.1-0.3, which was comparable to neem extract, followed by cow manure with a reproductive index of (0.2-0.3), green manure (0.2-0.4), pig manure (0.3-0.4) and chicken manure (0.3-0.5) compared to inoculated unamended control.

Table 4.4: Cyst numbers and reproductive index (RI) of potato cyst nematodes on different organic amendments

Organic amendment	Season one			Season two		
	Final cyst count (Pf)/pot	RI (Pf/Pi)	%decrease in cyst count from initial population(Pi)	Final cyst count(Pf)/pot	RI (Pf/Pi))	%decrease in cyst count from initial population(Pi)
Control	32.9 c	1.0 c		39.5 c	1.0c	
Neem extract	7.0 a	0.1 a	86	16.8 c	0.3ab	66
Green manure	9.9 ab	0.2 ab	80	18.7 ab	0.4ab	63
Chicken manure	13.5 b	0.3 b	73	22.8 b	0.5b	54
Pig manure	13.3 b	0.3 b	73	20.0 ab	0.4ab	60
Cow manure	8.0 a	0.2 a	84	12.5 a	0.3a	75
Goat manure	6.9 a	0.1 a	86	14.5 ab	0.3ab	71
S.E.M	1.5	0.03		3.0	0.06	
L. S. D	4.2	0.08		8.8	0.18	
CV (%)	12.3	12.3		34.9	34.9	

Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variance, LSD=Least Significant Difference, S. E. M= Standard Error of Means.). Initial population (Pi) = 50 cysts, reproductive index (RI=pf/pi).

4.6.2.4 Effects of organic amendment on viable eggs of potato cyst nematodes

Organic amendments significantly ($p<0.05$) reduced the reproduction of viable eggs to less than one compared to inoculated unamended pots in seasons one and two. The highest reproductive index of viable eggs was in inoculated unamended control with RI of 2.01-2.21. Cow manure had the highest decrease in the number of newly formed viable eggs among the organic amendment with RI of 0.3-0.42, followed by goat manure (0.6-0.18), pig manure (0.2-0.7), chicken manure (0.4-0.5) and green manure (0.9-0.04) compared to inoculated unamended control (Table 4.5).

Table 4.5: Reproductive index of potato cyst nematodes viable eggs on different organic amendments

Organic amendment	Season one		Season two	
	Final egg count(pf)/cyst	RI(Pf/Pi)	Final egg count(pf)/cyst	RI(Pf/Pi)
Control	201.2 c	2.01 c	221.7 c	2.21 c
Neem extract	27.7 a	0.27 a	15.2 ab	0.15 ab
Cow manure	31.0 ab	0.31 ab	41.7 ab	0.42 ab
Goat manure	61.2 ab	0.61 ab	17.7 ab	0.18 ab
Green manure	88.5 b	0.88 b	4.2 a	0.04 a
Chicken manure	39.2 ab	0.39 ab	54.8 ab	0.55 ab
Pig manure	15.2 a	0.15 a	72.8 ab	0.73 ab
S.E.M	23.2	0.23	20.8	0.21
L. S. D	66.6	0.66	59.8	0.60
CV(%)	16.3	16.3	15.2	15.2

Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means. Initial population (Pi) = 100 eggs, reproductive index (RI=pf/pi).

4.6.3 Field experiment

4.6.3.1 Effects of organic amendments on the growth (height, stem diameter) of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Treatments with organic manure in PCN-infested plots had no significant (p<0.05) difference in the growth of plants across potato cultivars and did not differ significantly from the unamended control. Potato cultivar Desiree in plots amended with green manure was taller (8.90-11.21 cm), followed by goat manure (7.89-8.32 cm) and cow manure (7.40-8.83 cm) compared to infested unamended plots. Potato cultivar Markies in plots amended with goat manure were significantly taller (11.49-14.93 cm), followed by green manure (10.96-13.51 cm) and cow manure (10.08-11.64 cm) compared to infested unamended plots. Potato cultivar Shangi in plots amended with green manure were taller (23.19-34.10 cm), followed by goat manure (20.10-30.39 cm) and cow manure (19.11-29.56 cm) compared to infested unamended control. Plants amended with organic manure recorded a higher number of stems between 2 to 3 across the cultivars planted compared to infested unamended plots. Potato cultivar Desiree in plots amended with green manure had wider stems (2.59-4.27 mm), followed by goat manure (2.51-3.23 mm) and cow manure (2.64-2.86 mm) compared to infested unamended control. Potato cultivar Markies in plots amended with green manure had wider stems (3.22-5.27 mm), followed by cow manure (3.40-4.9 mm) and goat manure (3.04-4.6 mm). Potato cultivar Shangi in plots amended with goat manure had wider stems (3.15-7.87 mm), followed by green manure (3.04-7.19 mm) and cow manure (3.11-6.93 mm) compared to infested unamended plots (Table 4.6).

Table 4.6: Effects of organic amendments on growth of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Variety	Treatment	Season one			Season two		
		Plant height (cm)	Stem no.	Stem diameter (mm)	Plant height (cm)	Stem no	Stem diameter (mm)
Desiree	Control	6.7 a	1.5 a	1.9 a	5.9 a	0.6 ab	1.7 a
	Cow manure	8.8 a	2.1 abcd	2.6 ab	7.4 ab	0.6 ab	2.9 ab
	Goat manure	8.3 a	2.1 abcd	2.5 ab	7.9 abc	0.9 abc	3.2 abc
	Green manure	8.9 a	2.4 cde	2.6 ab	11.2 abc	1.4 bcde	4.3 bcd
	Neem extract	6.6 a	1.6 ab	1.9 a	5.5 a	0.4 a	1.5 a
Markies	Control	9.6 a	1.8 abc	3.0 bc	9.8 abc	1.7 cdef	4.2 bcd
	Cow manure	10.1 a	1.8 abc	3.4 c	11.6 abc	2.5 fg	4.9 cd
	Goat manure	11.5 a	2.0 abcd	3.0 bc	14.9 c	1.1 abcd	4.6 bcd
	Green manure	11.0 a	1.8 abc	3.2 bc	13.5 bc	2.2 efg	5.3 de
	Neem extract	10.1 a	1.6 ab	3.0 bc	12.2 abc	2.0 defg	4.5 bcd
Shangi	Control	18.6 b	2.9 e	2.9 bc	23.5 de	2.9 g	6.0 def
	Cow manure	19.1 b	2.6 de	3.1 bc	29.6 def	2.4 fg	6.9 ef
	Goat manure	20.1 b	3.0 e	3.2 bc	30.4 ef	2.4 fg	7.9 f
	Green manure	23.2 b	2.4 cde	3.0 bc	34.1 f	2.1 efg	7.2 ef
	Neem extract	19.5 b	2.3 bcde	2.9 bc	22.9 d	2.3 efg	5.8 de
MEAN		12.8	2.1	2.8	16.0	1.7	4.7
	Variety (V)	4.0	0.5	0.4	3.8	0.7	0.3
L. S. D	Treatment (T)	2.5	0.4	0.4	4.2	0.4	1.2
	V*T	5.0	0.7	0.7	7.1	0.9	1.9
CV(%)		20.2	19.9	15.2	27.0	25.4	26.1

V*T denotes the least significant difference for the interaction of variety and treatments (organic amendments). Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference

4.6.3 Effects of organic amendments on root mass of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Potato cultivars Desiree, Shangi and Markies planted in plots amended with organic manure had higher root mass which did not differ significantly ($p<0.05$) from the control plots. Sixteen weeks after plant germination, potato cultivar Desiree in plots amended with goat manure increased in root mass by 50% followed by cow manure (40%) and green manure (15%) compared to unamended control. Potato cultivar Markies in plots amended with goat manure increased in root mass by 24% followed by cow manure (15%) and green manure (12%) compared to unamended control. Potato cultivar Shangi in cow manure significantly increased in root mass by 59% followed by goat manure (44%) and green manure (38%) compared to infested unamended control (Table 4.7 and 4.8).

4.6.3.3 Effects of organic amendments on yield of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Potato cultivars Desiree, Shangi and Markies planted in plots amended with animal and green manure had no significant difference ($p<0.05$) in tuber yield and did not differ significantly from the unamended control. Organic amendments significantly increased marketable (ware) and seed yields by 22 t/ha, followed by nonmarketable (chats) recording 19 t/ha across the potato cultivars. Potato cultivar Desiree in plots amended with cow manure increased in total yield by 28%, followed by goat manure (27%) and green manure (20%) compared to unamended control. Cultivar Markies in plots amended with green manure increased in total yield by 21% followed by cow manure (18%) and goat manure (17%) compared to unamended control. Shangi variety in plots amended with cow manure increased in total yield by up to 53% followed by green manure 36% and goat manure 30% compared to infested unamended control (Tables 4.7 and 4.8).

Table 4.7: Effects of organic amendments on yield and root mass of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes season one

Variety	Treatment	Seed (t/ha)	Ware (t/ha)	Chats (t/ha)	Total yield (t/ha)	Root mass (g)	%increase Root mass Over Control	%increase Yield over control
Desiree	Control	1.3 a	1.0 a	1.2 a	4.2 a	82.3 a		
	Cow manure	2.0 ab	1.7 ab	1.7 ab	4.7 ab	109.6 abc	33	12
	Goat manure	2.3 ab	1.7 ab	1.5 ab	5.2 ab	106.3 abc	29	24
	Green manure	1.7 ab	1.3 ab	1.7 ab	4.3 ab	86.8 ab	5	4
	Neem extract	1.3 a	0.8 a	1.2 a	4.3 ab	86.8 ab		
Markies	Control	2.0 ab	1.7 ab	1.3 ab	5.8 ab	157.1 abcd		
	Cow manure	2.8 b	1.7 ab	2.2 ab	6.8 ab	177.4 cd	13	17
	Goat manure	2.0 ab	3.0 c	2.0 ab	7.0 b	188.5 d	20	20
	Green manure	3.0 b	1.7 ab	2.7 ab	6.3 ab	188.0 d	20	8
	Neem extract	1.8 ab	1.7 ab	1.7 ab	5.8 ab	149.2 abcd		
Shangi	Control	1.7 ab	2.3 bc	1.3 ab	5.0 ab	129.4 abcd		
	Cow manure	2.3 ab	1.7 ab	2.2 ab	6.5 ab	162.4 bcd	26	29
	Goat manure	2.0 ab	1.7 ab	3.0 b	7.0 b	166.3 cd	29	20
	Green manure	1.8 ab	2.3 bc	1.7 ab	5.8 ab	141.2 abcd	9	17
	Neem extract	2.3 ab	1.7 ab	2.0 ab	6.0 ab	170.5 cd		
MEAN		2.0	1.7	1.8	5.7	140.1		
	Variety (V)	0.6	0.9	0.7	1.0	60.9		
L .S. D	Treatment (T)	0.9	0.7	1.0	1.7	38.6		
	V*T	1.4	1.2	1.7	2.7	75.8		
CV(%)		43.5	22.6	58.6	30.7	28.3		

V*T denotes the least significant difference for the interaction of variety and treatments (organic amendments). Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference.

Table 4.8: Effects of organic amendments on the yields and root mass of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes season two

Variety	Treatment	Seed (t/ha)	Ware (t/ha)	Chats (t/ha)	Total yield (t/ha)	Root mass (g)	%increase Root mass over control	%increase. Yield over control
Desiree	Control	1.5 a	1.0 a	2.3 bcde	4.2 a	58.90 a		
	Cow manure	1.8 a	1.8 ab	2.2 bcd	6.0 abc	86.60 ab	47	44
	Goat manure	1.8 a	1.5 ab	2.0 abc	5.3 ab	100.90 abc	71	28
	Green manure	2.0 ab	1.3 a	1.7 ab	5.7 ab	73.40 a	25	36
	Neem extract	2.0 ab	1.2 a	2.0 abc	5.2 ab	68.40 a		
Markies	Control	2.5 ab	4.0 c	2.0 abc	7.8 bcde	159.90 de		
	Cow manure	3.3 bc	4.0 c	2.0 abc	9.3 def	185.40 de	16	19
	Goat manure	3.3 bc	4.5 c	1.8 ab	8.8 def	204.20 e	28	13
	Green manure	4.0 c	4.5 c	1.3 a	10.5 f	164.30 de	3	34
	Neem extract	3.3 bc	4.0 c	2.3 bcde	10.2 ef	181.80 de		
Shangi	Control	2.2 ab	0.7 a	3.3 e	5.5 ab	88.40 ab		
	Cow manure	3.2 bc	3.2 bc	2.7 cde	9.7 def	170.00 de	92	76
	Goat manure	2.8 abc	2.2 ab	2.8 de	7.7 bcde	139.70 bcd	58	39
	Green manure	2.5 ab	4.0 c	2.0 abc	8.5 cdef	148.00 cd	67	55
	Neem extract	2.5 ab	2.0 ab	2.7 cde	7.2 bcd	131.40 bcd		
MEAN		2.6	2.7	2.2	7.4	130.8		
	Variety(V)	0.6	0.9	0.4	1.8	27.1		
L .S. D	Treatment(T)	0.8	1.0	0.4	1.5	32.6		
	V*T	1.3	1.7	0.7	2.6	54.0		
CV(%)		30.3	38.3	17.8	20.2	25.6		

V*T denotes the least significant difference for the interaction of variety and treatments (organic amendments). Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, RM=root mass.

4.6.3.4 Effects of organic amendments on potato cyst nematodes population density

There was no significant (*p*<0.05) difference in the initial PCN population in plots amended with organic manure and unamended control in seasons one and two. Treatments with organic amendments significantly (*p*<0.05) reduced final cyst population density across the potato cultivars and were statistically different from unamended control in seasons one and two (Table 4.9). Plots amended with cow manure had a maximum reduction in cyst population density by 82%, followed by goat manure 73% and green manure (70%) across potato cultivars Desiree, Shangi and Markies compared to infested unamended control. The least reproductive index was in goat manure (0.008-0.22), followed by cow manure (0.06-0.36) and green manure (0.14-0.37) compared to unamended control which had a reproductive index of more than one (Table 4.9).

4.6.3.5 Effects of organic amendment on viability of potato cyst nematodes eggs

Plots amended with organic manure significantly reduced the number of viable encysted eggs compared to infested unamended control. There was a significant (*p*<0.05) difference in newly formed viable eggs on plots amended with animal and green manure across the potato cultivars. A lower reproduction index was recorded in green manure (0.4-0.84), followed by cow manure (0.15-1) and goat manure (0.35-1.1), across the varieties, compared to infested unamended control (Table 4.10).

Table 4.9: Cyst numbers and reproductive index (RI) of potato cyst nematodes in different organic amendments

Variety	Treatment	Season one				Season two			
		Initial cyst Population(Pi) in 200 cm^3 soil	Final cyst Population(Pf) in 200 cm^3soil	RI (Pi/Pf)	%decrease in cyst count from initial population(Pi)	Initial cyst Population(Pi) in 200 cm^3 soil	Final cyst Population(Pf) in 200 cm^3 soil	RI (Pi/Pf)	%decrease in cyst count from initial population(Pi)
Desiree	Control	205.0 ab	398.0 cd	1.94ab		41.0a	933.0f	22.76d	
	Cow manure	296.0 abc	48.0 a	0.16 a	84	130.0ab	46.3abc	0.36a	64
	Goat manure	218.0 ab	48.0 a	0.22 a	84	219.0bc	78.0c	0.36a	64
	Green manure	387.0 abc	146.0 abcd	0.38 a	62	327.0c	52.3abc	0.16a	84
	Neem extract	279.0 abc	26.0 a	0.09 a		145.0ab	38.3abc	0.26a	
Markies	Control	488.0 bc	445.0 d	1.14 a		35.0a	353.0d	10.08b	
	Cow manure	369.0 abc	30.0 a	0.08 a	92	171.0abc	53.3abc	0.31a	69
	Goat manure	444.0 bc	105.0 abc	0.24 a	76	151.0ab	44.0abc	0.008a	71
	Green manure	319.0 abc	117.0 abc	0.37 a	63	180.0abc	34.3abc	0.19a	81
	Neem extract	308.0 abc	28.0 a	0.09 a		270.0bc	16.0abc	0.06a	
Shangi	Control	80.0 a	362.0 bcd	4.53 b		32.0a	440.0e	13.75c	
	Cow manure	277.0 abc	25.0 a	0.09 a	91	121.0ab	11.7ab	0.10a	90
	Goat manure	313.0 abc	84.0 ab	0.27 a	73	230.0bc	75.0bc	0.33a	67
	Green manure	254.0 ab	155.0 abcd	0.61 a	39	197.0abc	27.7abc	0.14a	86
	Neem extract	581.0 c	130.0 abc	0.22 a		150.0ab	7.7a	0.05a	
MEAN		321.0	143.0	0.7		160.0	43.8	0.6	
	Variety(V)	209.6	138.0	1.7		90.0	42.6	0.6	
L. S. D	Treatment(T)	179.7	178.1	1.4		102.2	38.8	0.8	
	V*T	317.7	308.5	2.6		172.0	63.6	1.3	
CV(%)		57.5	129.3	76.3		66.3	84.0	81.5	

V*T denotes the least significant difference for the interaction of variety and treatments (organic amendments). Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, initial cyst population-(Pi), final cyst population (Pf), reproductive index RI- (pf/pi).

Table 4.10: Effects of different organic amendments on reproductive index based on viable encysted eggs of potato cyst nematodes

Variety	Treatment	Season one		Season two	
		Final egg count(Pf)/cyst	RI (Pf/Pi)	Final egg count(Pf)/cyst	RI (Pf/Pi)
Desiree	Control	223.0 d	2.23 d	234.7e	2.35 e
	Cow manure	75.0 abc	0.75 abc	15.3a	0.15 a
	Goat manure	110.0 abc	1.10 abc	43.3abcd	0.43 abcd
	Green manure	40.3 ab	0.40 ab	84.0bcd	0.84 bcd
	Neem extract	15.3 a	0.15 a	92.0cd	0.92 cd
Markies	Control	323.0 e	3.23 e	210.0e	2.10 e
	Cow manure	15.3 a	0.15 a	27.7abc	0.28 abc
	Goat manure	57.7 ab	0.58 ab	4.7abcd	0.05 a
	Green manure	21.0 a	0.21 a	18.3ab	0.18 ab
	Neem extract	65.0 abc	0.65 abc	51.0abcd	0.51 abcd
Shangi	Control	160.0 cd	1.60 cd	228.0e	2.28 e
	Cow manure	100.3 abc	1.00 abc	53.0abcd	0.53 abcd
	Goat manure	134.0 bc	1.34 bc	34.7abc	0.35 abc
	Green manure	69.3 abc	0.69 abc	63.3abcd	0.63 abcd
	Neem extract	112.3 abc	1.12 abc	104.3d	1.04 d
MEAN		101.4	1.01	84.3	0.8
	Variety(V)	40.7	0.4	15.3	0.2
L. S. D	Treatment(T)	60.2	0.6	42.0	0.4
	V*T	97.4	0.97	65.9	0.66
CV(%)		61.0	61.0	51.3	51.3

V*T denotes the least significant difference for the interaction of variety and treatments (organic amendments). Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, initial population(Pi)=(100 viable eggs), reproductive index RI- (Pf/Pi).

4.7 Discussion

Findings from this study showed that organic amendments increased plant growth except for chicken manure under greenhouse conditions. Organic manure supplies nutrients and improves water-holding capacity, promoting the growth and development of plants (Van-Camp *et al*., 2004; Kankam *et al.,* 2015). In the process of decomposition, organic amendments re-mobilize phosphates and increase the availability of non-exchangeable potassium increasing nutrient cycling in the soil which positively influences plant growth (Hwang *et al.,* 2015; Solangi *et al.*, 2019). Mohammed and Alan. (2003), revealed that animal and green manures incorporated into the soil improve the performance of plants infested with nematodes. Chicken manure applied at the rate of 450 g/pot under greenhouse, resulted in shorter, thinner plants with reduced root mass compared to an unamended inoculated control. Rapid degradation of organic matter and production of short-chain volatile fatty acids in chicken manure are phytotoxic hence inhibiting the growth of potato plants under greenhouse conditions (Sasanelli *et al*., 2006; Renčo *et al*., 2011; Barje *et al.*, 2013). However, amounts of nitrogen content vary with the type of litter, type of bird and proportion of litter to droppings (Grandy *et al*., 2002). In this study, the findings on chicken manure are reflected by Bittsanszky *et al.* (2015), who reported phytotoxicity effects of chicken manure on plants due to high content of non-humified nutrients, which damages chloroplast, causes disturbance in photosynthesis and depletes carbon supply in the soil resulting in stunted plant growth and leaf chlorosis. Although findings by Sanguansak. 2004; Hou *et al.* (2012): Kankam *et al.* (2015); Karimipour Fard *et al.* (2019), showed that the application of chicken manure increased growth of tomato plants. Bulluck *et al.* (2002), reported that chicken manure increases the growth of tomatoes through the supply of nitrogen in the soil under greenhouse conditions.

Findings in this study showed that the organic amendments tested caused a reduction in final PCN population density, reproductive index and the proportion of viable eggs under greenhouse conditions. Animal manure (cow, goat, pig and chicken) increases the population of predatory micro-organism, which competes for space, water and food with PCN juveniles and eggs in the soil (Jatak, 2002). Animal manure during decomposition releases toxic ammonia, which alters soil structure, stimulates antagonist organisms against PCN cysts and eggs, improving plant tolerance to PCN (Lazarovits *et al.,* 2001). Various research has shown the successful control of root-knot nematodes by amending the soil with animal manure (Karmani *et al.,* 2011; Tanimola and Akarekor, 2014). Findings in this study are reflected by Abdel-Dayem *et al.* (2012); Osunlola and Fawole, (2015), who showed that animal manure had a toxic effect on *M. incognita* by releasing ammonium in the soil. The efficiency of animal manure depends on neutral pH, a high N content and a narrow C/N ratio. The green manure has glucosinolates compounds that break down to release isothiocyanate which has nematicidal effects on potato cyst nematodes population and egg viability (Dutta *et al.,* 2019; Motisi *et al.,* 2010; Matthiessen *et al.,* 2006). Findings in this study on the efficacy of green manure are in agreement with Lord *et al.* (2011), who showed that incorporation of green manure from brassica family in the soil was able to reduce cyst population density and caused over 95% mortality to encysted eggs of *Globodera pallida* under greenhouse conditions.

In this study, field experiment revealed that animal (goat and cow) manure and green manure increased plant growth parameters and tuber yield on potato cultivars Desiree, Shangi and Markies. Organic amendment (animal and green manure) improves soil structure, increases soil organic matter and nutrient content (Park *et al.,* 2011), enhances water holding capacity (Bulluck *et al*., 2002; Ros *et al.,* 2003) and stimulates soil microbial activity and plant growth (Van-Camp *et al*., 2004; Kankam *et al.,* 2015). In addition, soil fauna in animal and green manures contributes to the decomposition of organic matter, leading to mineralization of phosphorus and potassium, which enhances the development of a healthy root system by acting as a physical barrier to nematode movement to the roots hence enhancing plant growth (Hwang *et al*., 2015; Usman, 2015: Solangi *et al.*, 2019; Hellen, 2020). Organic amendments also supply nitrogen and enhance cat ion exchange capacity of phosphorus and potassium in the soil during plant growth (Oliveira *et al.*, 2010). These findings are reflected by Biratu *et al*. (2018), who reported that application of animal manure enhanced growth of cassava plants under field conditions. Hamm *et al.* (2016), showed that animal manure enhanced soil microbial activities, leading to improved soil productivity and plant growth. On the other hand, Schoebitz and Vidal. (2016), reported that amending the soil with animal manure improved the growth of perennial ryegrass. Manici *et al.*, (2018), reported an increase in the growth of tomatoes and zucchini after the incorporation of green manure into the soil to enhance nutrient availability and microbial growth promotion.

In this study, plots amended with animal and green manure increased the tuber yield of potato cultivars Desiree, Shangi and Markies under field conditions. Animal manure is rich in nitrogen, potassium and phosphorous, which enhance the development of root systems, rendering plants to be more tolerant to nematode attack significantly increasing tuber yield (Pakeerathan *et al.*, 2009; Thoden *et al.,* 2011). Oliviera *et al.*, (2010), reported that animal manure improved the commercial productivity of sweet potato tuber yield under field conditions. These findings are in concordant with McGuire, (2003), who showed that the incorporation of green manure in the soil was able to increase the tuber yield of potato crops under field conditions.

This study showed that animal and green manures significantly suppressed potato cyst nematodes population density and viability of PCN encysted eggs during crop development. Green manure (brassica species) in this study during decomposition was able to penetrate the hard cuticular layer of cysts, having an ovicidal effect on PCN juveniles which were vulnerable to this toxic volatiles emanated from decomposing brassica biomass (Dutta *et al.,* 2019). The ovicidal effect of brassica has also been demonstrated in the root-knot nematode, *Meloidogyne incognita* (Oliviera *et al.,* 2010). Findings in this study are reflected by Ngala *et al.* (2015), who observed that green manure from the brassica family was able to suppress PCN population densities when chopped and incorporated into the soil under field conditions. Decomposition of organic matter in cow and goat manure stimulates soil microbial biomass releasing biocidal substances such as ammonia which has nematicidal effects on potato cyst nematodes juveniles and viability of encysted eggs (Oka, 2010; Thoden *et al.,* 2011; Renčo, M., and Kováčik, P. 2012). Oka *et al.* (2010), reported that the application of organic manure with a narrow C: N ratio and high nitrogen content reduced the reproductive index of RKN.

Cow and goat manure enhanced the aggregation of soil particles by directly bounding the micro-aggregates into macro-aggregates, which restricted nematode movement (Pakeerathan *et al.,* 2009). These findings are in agreement with Pakeerathan *et al.* (2009), Tanimola and Akarekor. (2014), Osunlola and Fawole. (2015) and Maina *et al.* (2020), demonstrated that cow and goat manure were effective in reducing plant parasitic nematodes in sweet potatoes, tomatoes and okra under field conditions. Finally, both greenhouse and field results indicated that organic amendments (animal and green manure) have the potential in reducing PCN population density, reproductive index and viable encysted eggs, because they have a narrow C: N ratio and high nitrogen content (Jama *et al.,* 2000; Waceke, 2001; 2002).

CHAPTER FIVE

EFFICACY OF BIO-CONTROL AGENTS IN THE MANAGEMENT OF POTATO CYST NEMATODES UNDER GREENHOUSE AND FIELD CONDITIONS

5.1 Abstract

Potato cyst nematodes are a newly introduced pest in Kenya causing serious devastation to the potato crop. Several strategies are in place to manage PCN with the use of synthetic nematicides posing environmental concerns. Environmentally friendly strategies such as the use of botanicals and bio-control agents are recommended for PCN management. This study sought to determine the potential of commercial bio-control agents (*Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma hamatum*, *Trichoderma asperellum*, *Purpureocillium lilacinus* and *Bacillus subtillis*) in the management of PCN under greenhouse and field conditions. Chitted potato cultivar Shangi was treated with bio-control agents (BCAs) at the rate of 125 g/ha and sown in sterile sand-loam mixed at 2:1 (*w/w*) and 50 PCN cysts were introduced into each pot. A commercial neem-based product registered to control root-knot nematodes was included as a standard. Treatments were arranged in a completely randomized design replicated six times under greenhouse conditions and a split-plot design with potato varieties as the main plot and BCAs as the subplot under field conditions. Data collected was on plant growth parameters, cyst count and viable eggs. Under greenhouse, plants treated with BCAs had an increase in height and stem girth by 30.5 cm and 4 mm respectively. Plants treated with *Trichoderma* spp increased in root mass by 22.9 g while plants treated with *Purpureocillium lilacinus* and *Bacillus* reduced in root mass by 9.6 and 1.9 g respectively compared to control. Bio-controls significantly reduced cyst population density by 44 cysts compared to control. Under field, plants treated with BCAs had an increase in height, stem girth and root mass by 5.7 cm, 0.8 mm and 40 g respectively. Bio controls reduced cyst count by

96 cysts with a reproductive index of <1 compared to the control. Biocontrol agents increased total yield by 2.8 t/ha on all potato cultivars. This study revealed that BCAs have the potential of lowering PCN population density under greenhouse and field conditions.

5.2 Introduction

Potato (*Solanum tuberosum*) is the third most important food crop consumed globally after rice and wheat (CIP. 2019). In Kenya, the crop is the second most important staple food crop after maize, with over 800,000 small-scale farmers and 2.5 million people benefiting directly from the potato value chain (Abong and Kabira. 2013; CIP. 2019). In addition to that, potato is a rich source of carbohydrates, protein and mineral supply to the human diet (Birch *et al.*, 2012).

Potato cyst nematodes (*Globodera* spp), are pests of great economic importance to potato crops causing yield losses of up to 70% globally, which is equivalent to €300 million loss annually (Turner and Subbotin. 2013). In Europe, potato cyst nematodes cause up to a 9% reduction in potato production, equating to €43 million loss annually (Sasaki-Crawley. 2013). In Kenya, it is estimated that PCN causes up to a 61% decline in potato production, even with increased production area (FAO. 2017).

Symptoms of PCN infestation on potato plants are necrosis and stunted growth (EPPO. 2009). During an infestation, potato cyst nematodes juveniles in the infective stage (J2) enter the root cortex and move through the root tissue, dissolving the cell walls and causing secondary infections (Jones *et al.*, 2013). Once inside the plant tissues, potato cyst nematodes (J2) become sedentary, forming feeding tubes (Sobczak and Golinowski. 2011). During penetration and feeding of PCN in the plant tissues, the nematode secretes effector proteins that alter the host cell structure suppressing the host defense response (Haegeman *et al.*, 2012; Yang *et al.*, 2019).

Management of potato cyst nematodes revolves around the use of plant extracts, synthetic nematicides, genetic resistance, crop rotation, trap crops and organic manure, which have variations in efficacy against PCN, as the pest remains persistent in the soil threatening potato production (Ochola *et al.,* 2020). For instance, chemical nematicides such as Aldicarb (Temik), a carbamate have been done away with from use since they are toxic to aquatic life, birds and the environment in general (Khalil. 2013).

Bio-control agents such as *Fusarium* spp, *Paecilomyces* spp, *Bacillus* spp and *Syncephalastrum* spp are advocated to replace these chemical nematicides, for they are known to be environmentally friendly with no toxicity effects and ability to survive in the soil for a long period as they multiply rapidly under conducive environment (Khan *et al*., 2006). Various studies have shown that these bio-control agents normally parasitize plant parasitic nematodes together with their eggs (Anastasiadis *et al.*, 2008). Different strains of bio-control agents release secondary metabolites in the soil which effectively lower plant parasitic nematodes' population density (Sahebani and Hadavi. 2008; Hashem and Abo-Elyousr. 2011).

For instance, *P. lilacinus* parasitize various stages of plant parasitic nematodes hence effective against these nematodes (Khan *et al*., 2006; Anastasiadis *et al.,* 2008). Filamentous fungus *Syncephalastrum* spp has been effective against various PPNs in china (Sun *et al.,* 2008). *Syncephalastrum. racemosum* has been documented to reduce root-knot nematodes population density in soils resulting in an increased yield of cucumber (Sun *et al.,* 2008; Huang *et al.,* 2014).

Furthermore, *Bacillus* spp. evaluated under field conditions are known to produce large grey to white colonies with irregular margins under an unconducive environment, causing parasitism to plant parasitic nematodes (Dawar *et al.,* 2008). *Bacillus cereus* produces lytic enzymes, which are antagonists to *M. javanica* (Wepuhkhulu *et al.,* 2011). These BCAs exhibit different abilities to colonize nematode eggs when used individually (Huang *et al*., 2016). There is limited information on how BCAs interact with PCN and therefore, this study was conducted to determine the efficacy of *Trichoderma* spp, *Purpureocillium lilacinus* and *Bacillus subtillis* against potato cyst nematodes under greenhouse and field conditions in Kenya.

5.3 Materials and methods

5.3.1 Experimental study sites

Greenhouse experiments were conducted from March to May and June to August 2021 at University of Nairobi Upper Kabete Campus situated at 1° 15'S and 36° 44'E, with an elevation of 1,820 m above sea level (Sambroek *et al*., 1982). Field experiments were conducted during long and short rainy seasons from April to December 2021 in Nyandarua County-Murungaru sub-county situated (0.5676'S and 36.4689'E), at an altitude of 2384 m and receive 700-1500 mm of rainfall per annum.

5.3.2 Bio control agents and potato tuber selection

Commercial bio-control commonly used by farmers namely: *Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma hamatum*, *Trichoderma asperellum*, *Purpureocillium lilacinus* and *Bacillus subtillis*, were sourced from DUDUTECH and REAL IPM and applied according to manufacturer's recommendations. Certified seeds of potato cultivars Desiree (susceptible to PCN), Shangi (slightly tolerant to PCN) and Markies (tolerant to PCN) that are commonly grown by local farmers were sourced from the Kenya Agricultural and Livestock Research Organization-Tigoni.

5.3.3 Preparation of potato cyst nematodes inoculum

Potato cyst nematodes inoculum that was used for the greenhouse experiment was obtained from naturally infested fields in Nyandarua County. The Fenwick Can method (Fenwick, 1940) was used to extract PCN cysts from infested soil. The viability of the eggs within the cysts was verified by soaking a random sample of 10 cysts per soil sample in 0.001% Nile blue stain (*v/v*) for 48 hours (Faggian *et al.*, 2012). Dead eggs or juveniles stained blue while the live ones did not stain after opening the cyst. Samples with at least 50% of either live eggs or juveniles were used as sources of inoculum. The cysts were extracted and placed in Eppendorf tubes with each tube being used to preserve 50 cysts until needed.

5.3.4 Greenhouse experiment

Sandy-loam soil mixed at 2:1 (*w/w*) was sterilized by heating in an oven at 180° C in an oven in a 1 kg plastic pot after cooling. Chitted potato cultivar Shangi was sown into the pots. Fifty cysts were placed around the root rhizosphere 14 days after planting. *Trichoderma* spp were applied separately using a conidial suspension of 4.0×10^9 spores/ml; 20 g/l of water (125 g/2000 L /ha), *Purpureocillium lilacinus* was drenched with a conidial suspension of 1.0×10^8 spores/ml; 20g/l of water. *Bacillus subtillis* was applied at the rate of 2 L/ha around the plants' rhizosphere (Radwan *et al*., 2012). Negative control was unamended sandy-loam soil, while positive control was sandy–loam soil incorporated with Neem extract (6 L/ha). The experiment was laid out in a completely randomized design (CRD), with eight treatments each with four pots replicated six times and plants were watered after four days. At the end of the experiments, all soil was drenched with 10% (*v/v*) of sodium hypochlorite and allowed to settle for about five days before autoclaving for at least four hours to kill the cyst that remained after the experiment.

5.3.5 Field experiment

The field trial was conducted on a farm that was naturally infested with potato cyst nematodes in a split-plot design replicated three times, with the main plot being cultivars; Desiree, Shangi and Markies and subplots being the bio-control agents (*P. lilacinus*, *T. asperellum* and *T. atroviride*).

The bio-control agents were selected on basis of good performance in the greenhouse experiment. Potato seed tubers were soaked in *Trichoderma* isolates with a conidial suspension of 4.0×10^9 spores/ml; 20 g/l of water (125 g/2000 L/ha application rate) and *P. lilacinus* with a conidial suspension of 1.0×10^8 spores/ml; 20 g/l of water. Negative control was an untreated plot while positive control was plots treated with commercial neem extract at the rate of 6 L/ha. The experimental unit measured 9 m^2 with a spacing of 75 cm by 30 cm. Soil samples (200 cm^3) from each plot were collected before planting, at 45 days after planting and at harvest to determine cyst per 200 cm^3 and egg counts/cyst. The crops were rain-fed and kept free from weeds, pests and diseases through good agronomic practices during the growing season.

5.3.6 Potato cyst nematodes extraction.

Each soil sample from Nyandarua County was homogenized and a sample of 200 cm^3 of infested soil with potato cyst nematode was used to extract cyst nematodes using the Fenwick Can method (Fenwick, 1940).

5.4 Data collection

Data on plant growth parameters, final cyst count in 200 cm^3 of soil from each pot and viability test per cyst/pot were recorded 70 days after planting under greenhouse. Field data on plant growth parameters were determined 90 days after planting, while data on tuber yield/plot were recorded during harvesting. Total yield was further split into ware (>55 mm), seeds (25-55 mm) and chats (<25 mm) based on tuber diameter. Initial (pi)and final (pf) cyst population densities in 200 cm^3 of soil and egg viability of 3 cysts per treatment were determined at planting and harvesting respectively, followed by counting under dissecting microscope at 40x magnification.

5.5 Data analysis

Data on cyst and egg counts were normalized using square root transformation before analysis of variance (Gomez, 1984). The reproductive index of PCN cyst and viable eggs was expressed as RI = Pf /Pi (Van Den Berg and Rossing 2005). All the collected data on plant parameters, cyst counts and viable eggs were subjected to ANOVA test with Tukey multiple comparisons to determine statistical significance using GenStat software (15^{th} Edition). Treatments means were separated using least significant differences (LSD) tests at a 5% level of significance.

5.6 Results

5.6.1 Greenhouse experiment

5.6.1.1 Effects of bio-control agents on the growth of Shangi potato cultivar inoculated with potato cyst nematodes

There was significant (p<0.05) plant growth in the bio-control treatments compared to the untreated control. Amongst the bio-control treatments, a maximum mean plant height of 62.69 cm was recorded in plants treated with *T. asperellum,* followed by *T. hamatum* 60.18 cm, *P.lilacinus* (58.5 cm), *T. atroviride* (58.27 cm) and *T. harzianum* (56.5 cm), minimum mean plant height of 53.5 cm was recorded in plants treated with *Bacillus subtillis* over infested untreated control. The average number of stems during plant growth period ranged between 2 to 4 stems across BCAs treatments. Plants treated with *P.lilacinus* exhibited a wider mean stem girth of 3.66 mm, followed by *Bacillus* 3.57 mm, *T. hamatum* (3.44 mm), *T. harzianum* (3.41 mm) and *T. atroviride* (3.4 mm). Minimum mean stem girth was recorded in plants treated with *T. asperellum* (3.22 mm) compared to infested untreated control (Table 5.11).

5.6.1.2 Effects of bio-control on root mass of potato cultivar Shangi inoculated with potato cyst nematodes

There was a significant (p<0.05) difference in mean root weight in bio-control treatment compared to untreated control. Amongst the bio-control treatments, a maximum mean root weight of 35.46 g was obtained in plants treated with *T. harzianum*, followed by *T. asperellum* (28.54 g), *T. atroviride* (26.19 g), *P.lilacinus* (22.6 g) and *T. hamatum* (21.74 g). A minimum mean root weight of 13.96 g was obtained in plants treated with *Bacillus subtillis* (Table 5.1).

Table 5.11: Effects of bio-controls on the growth of potato cultivar Shangi infested with potato cyst nematodes

Treatment	Season one				Season two			
	Plant height (cm)	Stem number	Stem girth (mm)	Root mass (g)	Plant height (cm)	Stem number	Stem girth (mm)	Root mass (g)
Control	46.6 a	3.0 ab	3.4 bc	8.9 a	54.3 a	5.5 ab	3.3 a	16.2 a
T.asparellum	57.4 a	2.5 ab	3.0 ab	30.0 bc	68.0 b	4.0 a	3.5 ab	27.1 ab
T.atroviride	52.5 a	3.4 ab	3.3 bc	22.2 ab	64.1 b	4.2 ab	3.4 a	30.2 ab
P.lilacinus	49.1 a	2.2 ab	3.2 bc	20.1 ab	67.6 b	4.8 ab	4.2 b	24.2 ab
T.hamatum	54.6 a	3.1 ab	2.9 ab	16.4 ab	65.8 b	5.5 ab	3.8 ab	27.1 ab
T.harzianum	48.2 a	2.9 ab	2.5 a	22.9 ab	64.9 b	6.1 b	4.0 ab	48.0 c
Neem extract	62.9 a	4.2 b	3.3 ab	46.2 c	59.5 a	5.6 ab	3.6 ab	35.4 bc
B. subtillis	47.1 a	2.0 a	3.7 c	8.3 a	59.9 ab	4.1 a	3.5 ab	19.7 a
S.E.M	9.0	0.7	2.5	6.1	3.3	0.7	0.2	5.4
L. S. D	25.0	1.9	0.5	17.0	9.1	1.9	0.7	15.1
CV(%)	41.0	3.1	2.5	27.8	14.9	3.1	1.1	24.7

Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium. B-Bacillus.

5.6.1.3 Effect of bio-control agents on cyst population density

The bio-control agents tested caused a significant (p<0.05) reduction of final cyst population densities on potato cultivar Shangi compared to control. Maximum reduction in final cyst count was up to 86% in plants treated with *T.asperellum*, followed by *T.atroviride* (80%), *P.lilacinus* (79%), *T.harzianum* (76%) and *T.hamatum* (76%) compared to untreated control. The minimum reduction in final cyst count was 51% in plants treated with *B. subtillis* (Table 5.2). All the bio-control treatments had a reproductive index of less than one compared to untreated control where it was more than one

5.6.1.4 Effects of bio-controls on viable eggs of potato cyst nematodes

The reproductive index of viable eggs was immensely reduced to less than one compared to the untreated control with exception of *Bacillus subtillis.* There was a significant difference (p<0.05) in viable eggs on BCAs treatments in seasons one and two compared to untreated control (Table 5.3). The highest reduction in cyst reproduction index of 0.5 was in plants treated with *T. atroviride,* followed by *P.lilacinus* and *T. asperellum* each with a reproductive index of 0.3. *Trichoderma harzianum* had the least reproductive index of 0.2 compared to untreated control.

Table 5.12: Effect of bio-controls on potato cyst nematodes population density and reproductive index

Treatment	Season one			Season two		
	Final cyst count(Pf)/pot	RI (Pf/Pi)	% decrease in cyst count from the initial population(Pi)	Final cyst count(Pf)/pot	RI (Pf/Pi)	%decrease in cyst count from the initial population(Pi)
Control	53.0 d	1.06 d		48.3 c	1.0 c	
T.asperellum	5.6 a	0.11 a	89	9.1 a	0.18 a	82
T.atroviride	12.4 ab	0.25 ab	75	8.0 a	0.16 a	84
T.harzianum	12.7 ab	0.25 ab	75	8.9 a	0.18 a	82
T.hamatum	14.3 b	0.29 b	71	9.9 a	0.20 a	80
P.lilacinus	13.3 b	0.27 b	73	7.9 a	0.16 a	84
Neem extract	10.6 ab	0.21 ab	79	10.1 a	0.20 a	80
Bacillus subtillis	24.3 c	0.48 c	52	24.7 b	0.5 b	50
S.E.M	2.6	0.1		2.0	0.04	
L. S. D	7.4	0.2		5.6	0.11	
CV(%)	6.8	6.8		22.9	22.9	

Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means. Reproductive index (RI=Pf/Pi), initial cyst population (Pi)= 50 cysts, T-Trichoderma, P-Purpureocillium.

Table 5.13: Effect of bio-controls on number of viable egg of potato cyst nematodes

Treatment	Season one		Season two	
	Final viable egg count(Pf)/cyst	RI (Pf/Pi)	Final viable egg count(Pf)/cyst	RI (Pf/Pi)
Control	249.6 c	2.5 c	166.6 c	1.67 c
T.asperellum	94.4 ab	0.94 ab	12.4 a	0.12 a
T.atroviride	97.7 ab	0.98 ab	33.9 a	0.34 a
P.lilacinus	63.1 a	0.63 a	26.1 a	0.26 a
T.hamatum	86.3 ab	0.86 ab	30.9 a	0.31 a
T.harzianum	74.6 ab	0.75 ab	23.4 a	0.23 a
Neem extract	12.9 a	0.13 a	36.9 a	0.37 a
Bacillus subtillis	177.3 bc	1.77 bc	101.7 b	1.02 b
S.E. M	35.1	0.35	18.6	0.19
L. S. D	99.6	0.99	52.9	0.53
CV(%)	54.8	54.8	91.2	91.2

Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means. Reproductive index (RI=Pf/Pi), initial viable eggs (Pi)=100 eggs, T-Trichoderma, P-Purpureocillium.

5.6.2 Field experiment

5.6.2.1 Effect of bio-control on the growth of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Bio-control treatments had a significant increase in plant growth ($p<0.05$) across the varieties compared to untreated plants. Potato cultivar Desiree in plots treated with *T. atroviride* had a mean plant height of 8.15 cm, followed by, *T. asperellum* (7.81 cm) and *P. lilacinus* (7.4 cm). Potato cultivars Markies in plots treated with *T. asperellum* had a mean plant height of 13.08 cm, followed by *P. lilacinus* (11.95 cm) and *T. atroviride* (11.7 cm). Potato cultivar Shangi in plots treated with *T. atroviride* had a mean plant height of 30.12 cm, followed by *T. asperellum* (29.48 cm) and *P. lilacinus* (29.20 cm) and did not differ statistically from untreated control. Plants treated with bio-controls recorded number of stems ranging from 2 to 3 compared to control. Potato cultivar Desiree in plots treated with *T. asperellum* had a mean stem girth of 4.15 mm followed by *P.*

lilacinus (3.71 mm) and *T. atroviride* (3.68 mm). Potato cultivar Markies in plots treated with *P.lilacinus* had a mean stem girth of 5.37 mm, followed by *T. asperellum* (5.19 mm) and *T. atroviride* (5.17 mm). Potato cultivar Shangi in plots treated with *T. asperellum* had a mean stem girth of 8.62 mm, followed by *T. atroviride* (8.41 mm) and *P. lilacinus* (8.29 mm) and did not differ statistically with control (Table 5.4).

Table 5.14: Effects of bio-controls on the growth of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes

Variety	Treatment	Season one			Season two		
		Plant height (cm)	Stem Number	Stem girth (mm)	Plant Height (cm)	Stem number	Stem girth (cm)
Desiree	Control	9.2 a	2.8 b	4.4 ab	5.4 a	0.4 a	2.2 a
	Neem extract	8.5 a	2.9 b	3.9 a	5.3 a	0.6 a	2.8 a
	P.lilacinus	9.3 a	3.0 b	5.2 b	5.5 a	0.6 a	2.3 a
	T.asperellum	9.3 a	2.9 b	5.0 ab	6.4 ab	1.1 abc	3.3 a
	T.atroviride	9.8 a	3.0 b	5.0 ab	6.5 ab	0.8 ab	2.4 a
Markies	Control	9.3 a	2.9 b	4.7 ab	8.5 abc	1.6 bcd	3.3 a
	Neem extract	10.1 a	3.8 c	5.2 b	15.6 cd	2.7 e	5.6 bc
	P.lilacinus	10.4 a	3.2 cb	5.3 b	13.5 bcd	2.5 de	5.4 b
	T.asperellum	9.5 a	3.3 cb	5.1 ab	16.7 d	2.3 de	5.3 b
	T.atroviride	9.4 a	2.6 b	5.0 ab	14.0 bcd	2.1 de	5.3 b
Shangi	Control	27.3 b	1.6 a	9.0 c	24.6 e	2.1 de	6.9 bcd
	Neem extract	29.2 b	1.8 a	9.3 c	26.9 e	2.4 de	7.1 bcd
	P.lilacinus	28.1 b	1.8 a	9.1 c	30.3 e	2.3 de	7.5 d
	T.asperellum	33.0 c	1.8 a	10.0 c	25.9 e	2.6 e	7.3 cd
	T.atroviride	32.1 c	1.6 a	9.3 c	28.3 e	2.9 e	7.5 d
MEAN		16.3	2.6	6.4	15.6	1.8	4.9
	Variety(V)	1.4	0.3	0.7	5.1	0.7	0.9
L. S. D	Treatment(T)	1.8	0.5	0.7	4.4	0.5	1.1
	(V*T)	3.0	0.8	1.2	7.8	0.9	1.8
CV(%)		11.5	18.3	11.1	29.2	28.1	22.3

V*T represents the least significant difference in variety × treatment interaction. Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium.

5.6.2.2 Effect of bio-control on root mass of potato cultivars infested with potato cyst nematodes

Bio-control treatments had no significant difference in root mass across the potato cultivars. Desiree variety in plots treated with *T. asperellum* increased in root mass by up to 33% followed by *P. lilacinus* (22%) and *T. atroviride* (19%). Markies variety in plots treated with *P. lilacinus* significantly increased in root mass by up to 52% followed by *T. atroviride* (39%) and *T. asperellum* (34%). Shangi variety in *T. asperellum* increased in root mass by up to 25%, followed by *T. atroviride* (19%) and *P. lilacinus* (10%) compared to infected control (Table 5.5 and 5.6).

5.6.2.3 Effect of bio-control on yield of potato varieties infested with potato cyst nematodes

The bio-control treatments did not significantly increase tuber yield across the potato cultivars. Bio-control treatments recorded a higher marketable yield of 24 t/ha (ware) followed by non-marketable (chats) and seed each recording 22 t/ha across the potato cultivars. Potato cultivar Desiree planted in plots treated with *P. lilacinus* increased in total yield by up to 22% followed by *T. atroviride* 18% and *T. asperellum* (13%). Potato cultivar Markies planted in plots treated with *P. lilacinus* increased in total yield by up to 45%, followed by *T. asperellum* (40%) and *T. atroviride* (33%). Potato cultivar Shangi planted in plots treated with *T. atrovirid*e increased in total yield by up to 23%, followed by *T. asperellum* (15%) and *P. lilacinus* (6%) compared to control (Table 5.5 and 5.6).

Table 5.15: Effect of bio-control agents on yields of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes in season one

Variety	Treatment	Seed (t/ha)	Ware (t/ha)	Chats (t/ha)	Total weight(t/ha)	Root Mass(g)	%Increase in Root mass over control	%Increase in Yield over control
Desiree	Control	1.3 a	0.9 a	2.2 abc	3.5 a	66.7 a		
	Neem extract	1.5 abc	0.9 a	1.5 a	4.4 ab	1.5 abc		
	P.lilacinus	1.1 a	0.9 a	2.2 abc	4.3 ab	86.4 abc	30	21
	T.asperellum	1.3 ab	0.7 a	2.0 abc	4.1 ab	81.4 abc	22	16
	T.atroviride	1.7 abc	0.9 a	1.9 ab	4.4 ab	79.7 ab	19	26
Markies	Control	2.0 abc	1.3 a	2.0 abc	5.4 bc	107.1 abc		
	Neem extract	1.9 abc	1.7 a	2.8 cd	6.3 c	125.6 bcd		
	P.lilacinus	2.4 cd	2.0 bc	2.0 abc	6.7 c	132.1 cd	23	24
	T.asperellum	1.9 abc	2.2 c	2.4 bc	6.5 c	122.9 bcd	15	21
	T.atroviride	1.9 abc	1.9 ab	1.9 ab	5.6 bc	107.9 abc	0.7	3
Shangi	Control	2.2 bc	3.0 cd	3.3 de	8.5 d	167.3 de		
	Neem extract	3.0 de	3.0 cd	4.1 e	9.6 de	228.0 f		
	P.lilacinus	3.0 de	3.0 cd	2.6 bcd	8.5 d	195.9 ef	17	0
	T.asperellum	3.5 e	3.3 d	3.7 e	10.6 ef	217.6 ef	30	24
	T.atroviride	3.5 e	4.6 e	3.9 e	12.0 f	212.4 ef	27	41
MEAN		1.9	1.8	2.31	6.02	132.7		
	Variety(V)	0.6	1.1	0.5	1.4	38.7		
L. S. D	Treatment(T)	0.5	0.6	0.4	0.8	27.4		
	V*T	0.9	1.2	0.8	1.6	51.4		
CV(%)		28.8	31.4	19.4	13	21.2		

V*T represents the least significant difference in variety × treatment interaction. Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium.

Table 5.16: Effect of bio-control agents on yields of potato cultivars Desiree, Shangi and Markies infested with potato cyst nematodes in season two

Variety	Treatment	Seed (t/ha)	Ware (t/ha)	Chats (t/ha)	Total weight(t/ha)	Root weight(g)	% Increase in Root mass over control	%Increase in Yield over control
Desiree	Control	2.0 ab	2.0 ab	2.2 a	5.4 a	61.3 a		
	Neem extract	2.2 abc	1.9 ab	1.9 a	5.9 a	75.7 ab		
	P.lilacinus	1.9 a	1.5 a	2.0 a	6.3 ab	70.1 ab	14	17
	T.asperellum	2.0 ab	1.9 ab	2.0 a	5.9 a	88.4 abc	44	10
	T.atroviride	1.9 a	1.9 ab	2.2 a	5.9 a	72.5 ab	18	10
Markies	Control	3.7 ef	5.6 cde	2.0 a	6.9 abc	123.1 abcd		
	Neem extract	4.8 f	7.4 e	1.8 a	13.9 f	235.4 f		
	P.lilacinus	2.6 abcd	2.2 ab	2.0 a	11.3 ef	223.1 ef	81	64
	T.asperellum	3.0 abcde	4.1 bcd	3.9 b	10.9 def	190.0 def	54	59
	T.atroviride	3.3 cde	5.9 de	1.9 a	11.1 def	219.4 ef	78	62
Shangi	Control	3.1 bcde	3.1 abc	2.8 ab	9.1 bcde	153.1 bcdef		
	Neem extract	3.0 abcde	2.6 ab	2.8 ab	8.0 abcd	133.6 abcde		
	P.lilacinus	3.5 de	4.3 bcd	2.4 a	10.2 de	156.3 bcdef	2	12
	T.asperellum	2.8 abcde	4.1 bcd	2.8 ab	9.6 cde	181.9 def	19	6
	T.atroviride	3.3 cde	3.3 abc	2.8 ab	9.4 bcde	168.7 cdef	10	4
MEAN		2.6	3.1	2.1	7.8	143.5		
L. S. D	Variety(V)	0.7	1.8	0.8	1.7	69.1		
	Treatment(T)	0.7	1.3	0.6	1.9	48.4		
	V*T	1.1	2.5	1.1	3.1	91.2		
CV(%)		25.8	44.3	26.5	24.7	34.6		

V*T represents the least significant difference in variety × treatment interaction. Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium.

5.6.2.4 Effect of bio-control agents on cyst numbers of potato cyst nematodes

Bio-controls significantly ($p<0.05$) reduced the final cyst count across the varieties. Plots treated with *P. lilacinus* and *T. atroviride* reduced cyst population density by up to 95%, followed by *T. asperellum* (92%) across the potato cultivars compared to infested untreated control. PCN reproductive index on bio-controls was less than 1, with untreated control having the highest reproductive index of 1.19 (Table 5.7 and 5.8).

5.6.2.5 Effect of bio-control on the viability of potato cyst nematodes eggs

Bio-control had a significant ($p<0.05$) reduction in the number of viable encysted eggs compared to control. The highest cyst reproduction index was in untreated control with above 1. All the bio-controls had a lower reproductive index of less than 1 across the potato cultivars (Table 5.9).

Table 5.17: Effect of bio-control agents on cyst population density in potato cultivars Desiree, Shangi and Markies in season one

Variety	Treatment	Season one: Initial cyst count(Pi)in 200 cm^3 soil	Final cyst count (Pf)in 200 cm^3 soil	RI (Pf/Pi)	%Decrease in cyst count from initial population(Pi)
Desiree	Control	166.3 abc	122.7 ab	1.00 a	
	Neem extract	111.7 a	9.3 ab	0.08 a	92
	P.lilacinus	167.0 abc	8.4 ab	0.05 a	95
	T.asperellum	247.7 c	22.9 ab	0.09 a	91
	T.atroviride	235.0 bc	14.5 ab	0.06 a	94
Markies	Control	191.3 abc	71.3 ab	0.40 a	
	Neem extract	134.7 ab	6.4 ab	0.05 a	95
	P.lilacinus	110.7 a	4.8 a	0.04 a	96
	T.asperellum	106.3 a	11.5 ab	0.11 a	89
	T.atroviride	165.0 abc	7.3 ab	0.04 a	96
Shangi	Control	155.7 abc	163.3 b	1.05 a	
	Neem extract	148.7 abc	12.2 ab	0.08 a	92
	P.lilacinus	136.7 ab	20.0 ab	0.15 a	85
	T.asperellum	139.3 ab	12.3 ab	0.09 a	91
	T.atroviride	155.7 abc	18.2 ab	0.12 a	88
MEAN		158.1	33.7	0.9	
	Variety(V)	45.0	154.1	1.0	
L. S. D	Treatment(T)	63.6	57.5	0.6	
	V*T	103.3	157.3	1.1	
CV(%)		41.3	24.5	0.6	

V*T represents the least significant difference in variety × treatment interaction. Significant differences at (p<0.05) exist between means in the same column with a different letter or letters, CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium.

Table 5.18: Effect of bio-control agents on cyst reproductive index in potato cultivars Desiree, Shangi and Markies in season two

Variety	Treatment	Season two			
		Initial cyst count(Pi) in 200 cm^3 soil	Final cyst count(Pf) in 200 cm^3 soil	RI (Pf/Pi)	%Decrease in cyst count from initial population(Pi)
Desiree	Control	257.0 f	93.0 c	0.36 ab	
	Neem extract	74.3 abc	4.2 a	0.06 a	94
	P.lilacinus	116.0 cde	5.9 a	0.05 a	95
	T.asperellum	166.3 e	3.7 a	0.02 a	98
	T.atroviride	153.0 de	4.0 a	0.03 a	97
Markies	Control	123.7 cde	19.3 ab	0.16 a	
	Neem extract	97.7 bcd	1.5 a	0.02 a	98
	P.lilacinus	49.7 ab	0.7 a	0.01 a	99
	T.asperellum	19.0 a	1.9 a	0.10 a	90
	T.atroviride	26.7 a	2.0 a	0.07 a	93
Shangi	Control	123.0 cde	76.0 bc	0.60 b	
	Neem extract	108.7 bcde	5.8 a	0.05 a	95
	P.lilacinus	157.3 de	5.2 a	0.03 a	97
	T.asperellum	105.3 bcde	6.2 a	0.06 a	94
	T.atroviride	124.0 cde	3.5 a	0.03 a	97
MEAN		151.8	15.5	0.3	
	Variety(V)	33.9	22.2	0.2	
L. S. D	Treatment(T)	37.3	31.1	0.2	
	V*T	62.5	50.6	0.4	
CV(%)		4.6	21.7	4.7	

V*T represents the least significant difference in variety × treatment interaction. Significant differences at (p<0.05) exist between means in the same column with a different letter or letters. CV(%)=Coefficient of Variation, LSD=Least Significant Difference, S. E. M= Standard Error of Means, T-Trichoderma, P-Purpureocillium.

Table 5.19: Effects of bio-control on the reproductive index of encysted viable eggs in potato cultivars Desiree, Shangi and Markies

Variety	Treatment	Season one		Season two	
		Final egg count(Pf)/cyst	RI (pf/pi)	Final egg count(Pf)/cyst	RI (Pf/Pi)
Desiree	Control	139.3 e	1.39 e	188.3 f	1.88d
	P.lilacinus	12.7 ab	0.12 ab	25.0 a	0.25a
	T.asperellum	9.3 a	0.09 a	93.3 bcd	0.93bc
	T.atroviride	34.3 abc	0.34 abc	28.3 a	0.28a
	Neem extract	41.0 abc	0.41 abc	95.3 cd	0.95c
Markies	Control	180.0 e	1.8 e	131.3 de	1.31d
	P.lilacinus	59.3 abc	0.59 abc	21.7 a	0.22a
	T.asperellum	79.3 cd	0.79 cd	40.0 a	0.40a
	T.atroviride	13.7 ab	0.13 ab	27.7 a	0.28a
	Neem extract	24.3 abc	0.24 abc	36.3 a	0.36d
Shangi	Control	133.7 de	1.34 de	151.3 e	1.51d
	P.lilacinus	29.7 abc	0.29 abc	34.3 a	0.34a
	T.asperellum	70.3 bc	0.7 bc	42.3 ab	0.42ab
	T.atroviride	21.7 abc	0.22 abc	61.0 abc	0.61abc
	Neem extract	69.3 abc	0.69 abc	37.7 a	0.38 a
MEAN		57.9	0.58	63.7	0.64
	Variety(V)	20.1	0.2	32.2	0.32
L. S. D	Treatment(T)	37.3	0.37	3.0	0.03
	V*T	59.4	0.59	51.9	0.5
CV(%)		66.3	66.3	48.3	48.3

V*T represents the least significant difference in variety × treatment interaction. Significant differences at ($p<0.05$) exist between means in the same column with a different letter or letters CV(%)=Coefficient of Variation, L.S.D=Least Significant Difference, S. E. M= Standard Error of Means, Initial population (Pi)=(100 viable eggs), T-Trichoderma, P-Purpureocillium

5.7 Discussion

Results in this study showed that BCAs increased the growth of potato cultivar Shangi in sterilized soil under greenhouse. Bio-control agents may enhances nutrient uptake in the soil, which improves plant active uptake mechanism making the plants tolerant to biological and abiotic stresses hence increasing in growth of plants (Harman *et al.,* 2004). Rhizosphere colonization by BCAs induces photosynthetic activity and carbon absorption in the leaves hence leading to an increase in plant growth (Vargas *et al.*, 2009). These findings are reflected by Terefe *et al.* (2009) who showed that the application of *Bacillus* spp on infested tomato plants by *M. incognita* led to increased growth by 50 and a 91% reduction in galling in the roots under greenhouse conditions. Similar studies by (Küçük and Kivanç 2003), show that *Trichoderma* spp improved wheat plant growth and root mass under greenhouse conditions. Anastasiadis *et al.* (2008) showed that the application of *P.lilacinus* to control *M. incognita* increased the growth of cucumber plants under greenhouse conditions.

Considering the current research, BCAs significantly reduced the final PCN population density and reproductive index of viable eggs under greenhouse conditions. *P.lilacinus* had a direct hyphal penetration and parasitization on second stage juveniles and adult females, producing acetic acid, paecilotoxins and eucinostatins compounds, which inhibits the formation of syncytia and production of protease and Chitinase enzyme (Singh *et al.*, 2013). *Trichoderma* spp competes with PCN for nutrients and space in the host plants roots causing mycoparasitism and inducing systemic resistance to host plants (Hhmau *et al.,* 2015; Lombardi *et al.*, 2018). *Bacillus subtillis* produces lytic enzymes and protease which affect PCN cysts (Page *et al*., 2014; Chen *et al.,* 2015). Iturin and surfactin, negatively affect nematode survival (Castaneda-Alvarez and Aballay 2016). The findings are in agreement with Huang *et al.* (2014), who showed that treating tomato plants with

P.lilacinus before transplanting resulted in an 18% reduction in root-knot nematodes population density in the soil under greenhouse conditions. Dababat *et al.* (2006); and Goswami *et al.* (2008) showed that the application of *Trichoderma* spp reduced root galling by up to 30.8% in tomato plants. Basyony *et al.,* (2018) showed that *B. subtillis* significantly reduced the number of galls and reproductive index of viable egg of *M. incognita* by 81.1 and 89.5%, respectively in tomato plants.

Results under field trials during short and long rains showed that potato varieties Desiree, Shangi and Markies treated with BCAs improved plant growth. The bio-control agents produce growth-stimulating factors (growth regulator and plant hormones) and decrease the concentration of plant growth inhibitory substances in the soil which stimulates plant defense against biotic and abiotic stress hence improving plant growth (Hermosa *et al.,* 2010; Seenivasan, 2011). The BCAs enhance fertilizer use efficiency, which increases nutrient uptake through improved root growth as well as enhancing the availability of nutrients such as phosphorus in the soil (Lima-Rivera *et al.,* 2016). Mostafa *et al.* (2015), showed that *P.lilacinus* improved potato growth and tuber yield of potato plants under field conditions. Olabiyi *et al.*, (2013) and Ferweez and Abd El-Monem. (2018) reported that the application of *Trichoderma* spp increased the growth and yield of soybean under field conditions.

Bio-control agents significantly reduced PCN final population density and reproduction of viable encysted eggs across potato cultivars Desiree, Shangi and Markies. *Trichoderma* spp exhibits chitinolytic activity, which causes degradation in the chitin layer of nematode cysts and eggs, increasing the accumulation of hydrolytic enzymes which affects the infective J2 stage of PCN from invading the roots of the host plant (Selim *et al.,* 2014). *P.lilacinus* directly infects the sedentary stage of PCN eggs in the soil lowering the reproductive index and final PCN population in the soil (Kiewnick and Sikora. 2006). During the infection process, *P.lilacinus* releases nematicidal compounds such as acetic acids and chitinases, which negatively affect PCN cysts and viable eggs (Park *et al.*, 2004). Bio-control agents colonize the root system, hence increasing the levels of plant defense enzymes, which induces systemic resistance to the host plant against PCN attack in the soil (Harman *et al.*, 2004; Gomes *et al.*, 2015). Findings by Osman *et al.* (2018) show that *Trichoderma* spp and *P.lilacinus* significantly reduced root-knot nematode *M. incognita* in eggplant under field conditions. Therefore, the application of *P.lilacinus* and *T. asperellum* significantly lowered the reproductive index of PCN cysts and viable eggs and hence should be considered in managing the pest.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General discussion

Generally, findings on present research showed that organic amendments and biocontrol agents had a positive impact on the growth of potato cultivars Desiree, Markies and Shangi. Organic manure improved soil structure, soil organic matter and nutrient content, enhancing water holding capacity and stimulating soil microbial activity which contributes to the degradation of organic matter in the soil enhancing cat ion exchange capacity releasing phosphorous, nitrogen and potassium which is essential in the development of healthy root system (Van-Camp *et al*., 2004; Oliveira *et al.,* 2010; Kankam *et al.,* 2015; Hwang *et al*., 2015; Usman, 2015; Solangi *et al.*, 2019; Hellen, 2020). Bio-control agents enhanced the uptake of nutrients in the soil and water use efficiency that improves plant active uptake mechanism making the plants tolerant to biotic and abiotic stress hence increase in plant growth (Harman *et al.,* 2004). Anastasiadis *et al.* (2008) and Biratu *et al*. (2018) reported similar results in cucumber and cassava respectively. The amount of potted soil, which is sandy loam soil at the ratio of 2:1 in a 4kg pot was inadequate to balance ammonia produced by chicken manure applied at the rate of 450g/pot, leading to the production of short volatile fatty acids which were Phytotoxic to potato plants (Sasanelli *et al*., 2006; Renčo *et al*., 2011; Barje *et al.*, 2013).

Secondly, the application of organic amendment and bio-control agents increased the root mass and tuber yield of potato cultivars Desiree, Markies and Shangi. Macronutrients available in organic manures (nitrogen, potassium and phosphorous), enhanced root growth rendering plants to be more tolerant to plant parasitic nematodes infestation leading to increased tuber yield (Pakeerathan *et al.,* 2009; Thoden *et al.,* 2011). On the other hand, bio-control agents produced growth stimulating factors such as growth regulators and plant hormones, as they decreased the concentration of plant growth inhibitory substances in the soil which stimulates plant defense against biotic and abiotic stress hence improving tuber yield of potato crop (Harmosa *et al.,* 2010; Seenivasan, 2011). Similar results on this study is reflected by Oliviera *et al.* (2010) and Mostafa *et al.* (2015) on the positive effects of organic manures and bio-control agents on tuber yield of sweet potato and Irish potatoes respectively.

Finally, findings in this study showed that the use of resistant potato cultivars, organic amendments and biocontrols reduced the reproductive index of the initial cyst population to final cyst population density and viable encysted eggs. Resistant potato cultivars had H3 resistance gene, which prevented the J2 from molting to the adult stage (Grunewald *et al.,* 2009; Varypatakis *et al.,* 2020). Organic manure released toxic volatiles, which stimulate soil microbial biomass, which had nematicidal effects on PCN juveniles and egg viability (Oka, 2010; Thoden *et al.,* 2011; Renčo, M., and Kováčik, P. 2012). Bio-control agents colonized the root mass increasing levels of plant defense enzymes, which induces systemic resistance to host plants against PCN attack in the soil (Harman *et al.,* 2004; Gomes *et al.,* 2015). Urek *et al*., (2008); Tanimola and Akarekor (2014); Osman *et al.* (2018), recorded similar results on plant parasitic nematodes (*Meloidogyne* spp).

6.2 Conclusion

In testing for the reaction of potato varieties to potato cyst nematodes, potato breeders need an effective test to screen numerous clones for tolerance to potato cyst nematodes. The application of animal and green manure at the rates of 20 t/ha and 45 t/ha respectively and bio-control agents especially the *Trichoderma* species and *P.lilacinus* had an overall increase in growth of Desiree, Shangi and Markies potato varieties compared to unamended/untreated pots/plots under greenhouse and field conditions. There was an overall increase in the yield of potatoes recorded on organic amendments and BCAs treatments. *Bacillus* (Regain) did not significantly ($p<0.05$) lower the final population cyst population (Pf).

6.3 Recommendation

I. Farmers should practice the use of organic amendments such as cow, goat, pig, chicken manures as well green manure at the rates of 20t/ha and bio-control agents such as *Trichoderma* spp and *P.lilacinus* at the rates of 125g/ha in the management of potato cyst nematodes both under greenhouse and field conditions.

II. Continuous research should be undertaken on the resistance status of the local potato cultivars to potato cyst nematodes and capacity build farmers on use of resistant/tolerant potato cultivars in the management of potato cyst nematodes.

III. There is a need for further studies to understand the efficiency of combining resistant potato cultivars, organic amendments and commercial bio-control agents as a package under field conditions

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