DIVERSITY, FACTORS INFLUENCING SPREAD AND POPULATION BUILD UP OF POTATO CYST NEMATODES AND POTENTIAL OF PHYTOCHEMICALS IN THEIR MANAGEMENT IN KENYA

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DECLARATION

This is my original work and has not been presented for an award of any degree in any other university

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This thesis is dedicated to my late dad Joseph Johnson N. Mbiyu who regrettably did not live to see this work, he encouraged us to live our dreams, my dearest mom Mary Njeri Mbiyu, and to my nephews Victor, Emmanuel Wattson, Frank, Sammy, Njeri, Geovanny, Joan and baby Allan for believing in me and trusting my decision to pursue PhD studies.

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LIST OF ABBREVIATIONS

AEZS	Agro-ecological zones
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
CRD	Completely randomized design
CL	Confidence limit
DAP	Diammonium phosphate
DNA	Deoxyribonucleic acid
dNTP s	Deoxynucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
ICIPE	International Center of Insect Physiology and Ecology
IPM	Integrated Pest Management
ITS	Internal transcribed spacer
J2	Second stage juvenile
J3	Third stage juvenile
J4	Fourth stage juvenile
KALRO	Kenya Agricultural and Livestock Research Organisation
KEPHIS	Kenya Plant Health Inspectorate Service
LC	Lethal dose
LSD	Least Significant Difference
Min	Minute
mtDNA	Mitochondria Deoxyribonucleic Acid
MoALF	Ministry of Agriculture, Livestock and Fisheries
NPCK	National Potato Council of Kenya
PCN	Potato cyst nematode
PCoA	Principal coordinate analysis

PCR	Polymerase chain reaction
Pf	Final population
Pi	Initial population
PLRV	Potato leaf roll virus
PIC	Polymorphic information content
PPN	Plant parasitic nematodes
PRD	Potato root diffusate
RCBD	Randomized complete block design
rDNA	Ribosomal DNA
RF	Reproductive factor
RI	Reproductive index
SSR	Simple sequence repeats
TAE	Tris Acetate EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultraviolet

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GENERAL ABSTRACT

Potato cyst nematodes (PCN) of the genus *Globodera* are one of the most significant constraints to potato production in Kenya. Limited research has been undertaken on the causal agent, practices that influence population build-up and spread of the pest as well as the economic importance of PCN populations in the country. Therefore, this study was carried out between 2019 and 2022 with the aim of determining the diversity of PCN populations in the country; site specific factors that contribute to spread and build-up in population density; relative yield loss caused by the pest; and the efficacy and active ingredients of selected botanical pesticides in PCN management. The diversity of potato cyst nematode populations was determined using morphometrical and simple sequence repeats markers. Morphometric analysis proved ineffective in differentiating *Globodera. pallida* from *Globodera rostochiensis*. Results of the molecular analysis showed that all the samples from the three study counties (Nyandarua, Nakuru and Meru) gave amplicons of 434bp that are specific for *G. rostochiensis.* The results showed that *G. rostochiensis* populations from the three counties were successfully discriminated by Gr 67, Gr79, Gr90 and GRM2 SSR markers (PIC ≥ 25). The fixation index (*F*st) ranged from 0.021 to 0.048 indicating that the samples had low genetic variation. To determine the factors contributing to spread and population build-up of potato cyst nematodes (*Globodera* spp.) in Nyandarua county, a survey was carried out in Upper Highland 3 (UH3), Upper Highland 2 (UH2) and Lower highland 4 (LH4). A total of 65 randomly selected farmers were interviewed using a structured questionnaire and soil samples collected. Results showed that cyst counts were higher (302 cysts/ 300 g) in farms with more than one crop cycle compared to farms where one crop cycle was grown per year having 38.5 cyst/300 g soil. Over 80% of the farmers used farm saved seed tubers and grew cultivar Shangi that is susceptible to PCN. The study showed that cysts adhered on farm tools, seed potato and foot wear. Assessment of the relative yield loss caused by potato cyst nematodes at varying infestation levels was determined through field experiments. Potato cyst nematodes reproduced more on Desiree and Shangi varieties than on variety Manitou indicating that the two are suitable PCN hosts. Tuber yield losses were significantly reduced in potato varieties treated with PCN control products over the untreated control plots. A positive correlation (r= 0.4640, r= 0.4802) ($p \leq 0.05$) between final cyst counts and reproduction index during long and short rains respectively was observed. Additionally, final cyst counts were significantly ($p \leq 0.05$) and negatively (r=-0.4987, r=-0.5085) correlated with total tuber yield during long and short rains season. Assessment of the efficacy of

selected botanical pesticides in potato cyst nematode management was done through *in vitro*, screenhouse and field experiments. Plant extracts from Mexican sunflower, garlic, ginger, Mexican marigold, spring onion, sodom apple, eucalyptus leaves, eucalyptus bark, green tea leaves and onion bulb were effective in inhibiting egg hatch, reducing egg viability, and increasing mortality of second stage juveniles (J2s). Application of hexane extracts from ginger, garlic and Mexican sunflower resulted in a significant ($P<0.05$) increase in juveniles mortality and loss of egg viability resulting in potato yield increase under screenhouse and field conditions. The ginger extract, at a concentration of 100 mg/ml, had an inhibitory effect on multiplication of the nematodes and was rated as the most potent in reducing numbers of PCN. Results from the survey work led to the conclusion that *G. rostochiensis* was the predominant species within the study area with limited genetic variation among the populations studied. Factors that were contributing to spread and build-up of potato cyst nematodes include cropping practices and soil types. Mexican sunflower, ginger, and garlic extracts have potent nematicidal effects against PCN J2s and eggs due to phytochemicals present in the extracts. The three botanical extracts significantly increased potato yields by 112.2 and 80.6 % during the short and long rains, respectively. Ginger extract, applied at 100 mg/ml is recommended as the most effective extract against PCN in order to improve potato yield. There is need for an integrated approach incorporating all appropriate strategies such as planting of disease-free tubers, resistant varieties, field sanitation as well as enhancing farmers' knowledge on management of the pest to reduce the spread and build-up of PCN.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum,* L.) is ranked as the fourth most important food crop in the world (Agricultural Marketing Resource Centre, 2018). The crop is grown for food and cash by both small and large-scale farmers, in Kenya. The crop is ranked second after maize in terms of national food security and poverty alleviation (MoALF, 2016). In addition, the potato industry provide income to about 3.3 million people along the value chain that includes producers, marketers, transporters, processers, vendors, retailers and exporters (Janssens *et al.,* 2013). Potato production is estimated to be 2,107,824 T, which is about KSh. 50 billion at farm gate prices, and produced on an estimated area of 214,600 ha per year (FAOSTAT, 2021, MoALF, 2021).

Despite the importance and large production area of potato, the yield has remained low at 9.8 t ha^{-1} compared to the national potential yield of 40 t ha^{-1} (FAOSTAT, 2021, MoALF, 2021). The major constraints contributing to the low yields include biotic stresses (insect pests, diseases and weeds), abiotic stresses (inadequate rainfall and low soil fertility), poor agronomic practices, and lack of resources (Muthoni, *et al.,* 2013). One of the main biotic factors limiting potato production is potato cyst nematodes (Deliopoulos *et al.*, 2010; Haukeland, 2016).

Potato cyst nematode (PCN) is an emerging pest in East Africa having been detected in Kenya in 2014 (Mwangi *et al.,* 2015) and in 2018 in Uganda and Rwanda (Niragire *et al.,* 2019). The two main PCN species; *Globodera rostochiensis* and *Globodera pallida,* reported to infect potato account for 9% of crop loss worldwide (Turner and Stone, 2019). Cysts contain 200 to 600 eggs each and are hardy and long-lived, surviving for at least 30 years without a host, making pest control difficult. Eradication is quite difficult after PCN has infected a field (Nieri and Karuri, 2018). Potato cyst nematode is a soil-borne pest that is spread through infected tubers as well as with infested soil adhering onto farm tools and equipment (EPPO, 2017). Thus, use of pathogen free planting material and washing of farm equipment's is paramount. However, access to quality seed is a major challenge for most of the smallholder farmers with 90 % of them using their own farm-saved seed or seed sourced from informal channels (Janssens *et al.,* 2013). Such seed is often of unknown quality and easily spreads diseases and pests including PCN.

In addition, the average farm size is less than 0.5 ha for the majority of potato growers which limits use of crop rotation as an option in pest and disease management (Muthoni *et al.,* 2013). Under rain fed conditions, it is possible to grow 2-3 potato crops on the same plot of land every year. It is estimated that continuous cultivation of potato in PCN infested fields may result in yield losses of up to 80% threatening the food security in the country (Haukeland, 2016). Moreover, potato is usually produced under monoculture thus enhancing PCN population build-up.

Worldwide, management of PCN is via use of integrated pest management (IPM) approaches which includes use of resistant varieties, cultural practices and nematicides (SASA, 2010). In Kenya, there are few varieties such as Rumba, Manitou and Caruso reported to have partial resistance to PCN however, they have breeders rights (KEPHIS, 2018) which restricts their availability to registered farmers only. While use of chemical pesticides is effective and fast acting they are too expensive with pertinent environmental challenges associated with their use (Adegbite and Agbaje, 2007).

1.2 Problem statement

Potato is of economic importance in Kenya as source of food and income. Potato cyst nematode (*Globodera spp*) is one of the major constrains to potato production. The pest has spread to nearly all major potato growing counties in Kenya and contribute to low potato yields of less than 10 t ha^{-1}. Once the pest infests a farm it can persist for over 30 years. This presents a significant threat to the sustainability of Kenyan potato production.

Despite the threat posed by the pest, there is scarcity of data on the genetic diversity of populations found in potato growing counties in the country. Nonetheless, the latter is critical in developing appropriate management strategies for the pest including development of varieties resistant to the pest. Until now, most of the research has focused on determining the extent of spread of the pest (Haukeland, 2016), development of rotational programs to support the natural decline of PCN in infested soils (Mburu *et al*., 2018), efficacy trials on new nematicide products (KALRO, 2018) and use of trapping technology in management of the disease (Chitambo, 2019).

Although the prevalence of PCN in the country has been mapped, information on area-specific factors such as host, environmental factors as well as farmers' practices that influence population build-up and spread of the pest is yet to be established. Such information is key in developing site-specific control strategies to manage the pest. Similarly, information on the yield losses caused by the pest is also limited yet such information is necessary for policy formulation and prioritization of the research and extension agenda on pests affecting potatoes.

Use of synthetic nematicides is one of the main means of management of plant parasitic nematodes. However, concerns about environmental effects of chemical control of PCN and associated costs for smallholder farmers limit the use of this approach. Consequently, alternative approaches such as the use of botanical pesticides are required to manage the pest. However, little is known about their efficacy under current climatic conditions.

1.3 Justification

The empirical evidence on the extent of genetic diversity of PCN populations in Kenya as well as site specific factors that influence the spread of the pest, the yield losses associated with the pest as well as efficacy of selected botanical pesticides will contribute to sustainable production of potatoes in Kenya. This will ultimately contribute to the national objectives of food security diversification and commercialization.

Findings on the genetic diversity of PCN populations will aid in better understating of the pest and will go a long way towards development of potato varieties tolerant to the PCN. Results from studies on area-specific factors contributing to the rapid spread and build-up of PCN population density will be key in aiding technology developers in the design and formulation of effective site-specific IPM control strategies to management of the pest.

Findings on yield losses due to PCN will be essential in informing and enhancing both public and private agricultural stakeholder, on the need to mainstream resources and capacity in an attempt to address the impact of PCN on potato production. Results from studies on the efficacy of botanical pesticides will go a long way in informing formulation of crop protection advisories that are not only cheaper but also environmentally friendly and safe for farmers and consumers of potatoes.

1.4 Research objectives

1.4.1 Broad objective

To reduce losses caused by PCN on potatoes through elucidation of their diversity and factors that influence PCN establishment and development of more sustainable management options

1.4.2 Specific objectives

The specific objectives were to:

i. To determine the diversity of PCN populations in Nyandarua, Nakuru and Meru counties
ii. To determine the site-specific factors that contribute to prevalence and population density of PCN in different agro-ecological zones (AEZS) in Nyandarua county
iii. To assess relative yield losses caused by PCN at varying PCN population densities
iv. To determine the efficacy and active ingredients of selected botanical pesticides in PCN management

1.5 Hypotheses

i. The genetic variability of PCN populations from Nyandarua, Nakuru and Meru counties is not significant.
ii. Site specific factors have no significant effects on prevalence and population density of PCN in the different agro-ecological zones in Nyandarua county.
iii. There are no significant differences in the yield losses caused by PCN at varying PCN soil densities.
iv. There are no significant effects of botanical extracts on the loss of PCN egg viability, inhibition of PCN egg hatch and mortality of juveniles.

CHAPTER 2: LITERATURE REVIEW

2.1 Potato (*Solanum tuberosum*)

2.1.1 History

Potato (*Solanum tuberosum* L.) was first cultivated in Southern America, specifically in Peru and Bolivia about 8000 years ago (Ugent *et al.,* 1987). After domestication, the crop spread through Chile, Colombia and Ecuador and arrived in Europe around 1590's and spread in European countries and further to African countries (Strowbridge, 1980; Devaux *et al*., 2014). In the 18th - 19th century, the European population increased drastically which led to over dependence on potato in European countries. However, the potato crop did not survive for several consecutive years due to a huge outbreak of the late blight disease. This catastrophe led to the Irish potato famine that resulted into deaths of a million people due to starvation (Ortiz and Mares, 2017). In Kenya, potato has been grown since the 1880s and since then it has gained importance as a major food and cash crop (Sophie, 2018).

2.1.2 Production and economic importance

Globally, potato serves as cash, food and nutrition security crop (Devaux *et al*., 2021). Africa produces about 7 % of the global production, Kenya being among the six biggest potato producer in Africa after Algeria, Egypt, Malawi, South Africa and Rwanda (Devaux *et al.*, 2014; FAOSTAT, 2021). In Kenya, potatoes are grown for food and as a source of income, contributing approximately KSh. 50 billion per year (Janssens *et al.*, 2013; MoALF, 2021). However, the yield has been declining in the recent years ranging from 20.3 t ha^{-1} in the year 2012 to 9.8 t ha^{-1} in the year 2021 (Figure 2.1), while potato yield in developed countries is 40 t ha^{-1} (MoALF, 2021). Majority of potato producers are small-scale farmers who own less than 1 acre of land, leading to a continuous production of potato in the same fields. This has led to a rapid build-up of PCN resulting into negative impacts on production thus affecting the total value of the crop. Potato is a short growth duration crop, with high nutrition value, high production per unit area (Janssens *et al.,* 2013). Its adaptability to many traditional diets as well as changes in dietary habits have contributed to rapid expansion of potato production (Sophie, 2018).

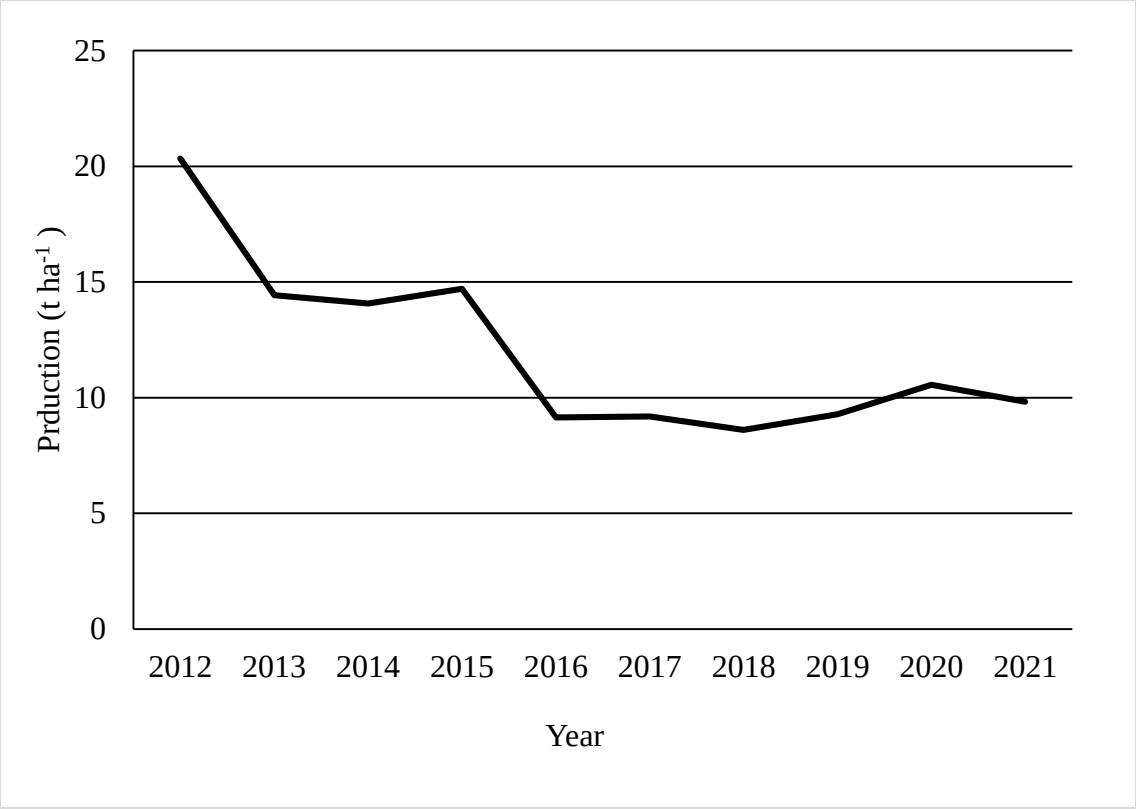

Table 2.1. Trends in potato production in Kenya (FAO, 2021)

In Kenya, potato is mainly cultivated in mid and high rainfall areas, in altitudes ranging between 1500-3000 metres above sea level (MoALF, 2021). These areas include both sides of Aberdare ranges such as Nyandarua, Nyeri and Kiambu counties; the slopes around Mt. Kenya such as Laikipia, Nyeri and Meru counties; the highlands of Mau Escarpment including Nakuru, Bomet, Narok and Kericho counties; areas around Nandi and Cherangani hills, Mt. Elgon, Kisii highlands, Taita hills and Elgeyo Marakwet highlands (MoALF, 2021). Due to the favourable weather in the highlands, farmers can complete three planting cycles in a year unlike maize (Muthoni and Nyamongo, 2009). Additionally, growth in population over time demands for more food leading to intensification of production and expansion of production to zones previously dominated by maize (Muthoni *et al.*, 2013).

2.1.3 Botany, biochemical composition and nutritional value

Potato belongs to the *Solanaceae* family (Hirano, 2015). Cultivated potatoes *Solanum tuberosum* (L.) are divided into different ploidy levels which include diploid (2n = 24), triploid (3n= 36),

tetraploid (4n= 48), pentaploid (5x= 60) (Hirano, 2015). Majority of cultivated polyploids in the world are tetraploid (4x= 48 chromosome), which has two sub species *Solanum tuberosum* and *Solanum andigena* (Patil *et al.*, 2016). It has roots that extend to about 40-50 cm and the tubers are enlarged underground stems adapted to store carbohydrates and for vegetative reproduction. The tubers originate from the tips of the underground stems called stolons that contain all characteristics of the normal stems including buds formed at the base of a leaf with detectable scars (Muthoni *et al.*, 2012).

Potatoes are rich in carbohydrates, which is more than 95% of the potato by weight. Significant amounts of vitamins and minerals can be found in potatoes, which include vitamin C, potassium, iron, phosphorous and magnesium. Depending on the cultivar, potatoes have a low protein content that ranges from 1 to 1.5% of their fresh weight. The protein from potatoes is of high quality because of its high digestibility and amino acid concentration. As compared to Potatoes contain less fibre than whole-grain cornmeal (7.3 g/100 g), but more fibre than white rice (0.3 g/100 g) or whole-wheat cereal (1.6 g/100 g). When compared to white rice (0.3 g/100 g) and whole-wheat cereal (1.6 g/100 g), potatoes have more fibre. This was recorded by Horton (1987) where cooked potatoes without the skin contain 1.8 g of fibre per 100 g, whereas cooked potatoes with the skin contain 2.1 g of fibre per 100 g.

2.2 Potato cyst nematode

2.2.1 Origin and distribution

Potato cyst nematodes were initially found to occur in Andean mountain in South America where they have co-evolved with *Solanaceae* hosts (Sullivan *et al*., 2007). In the middle of the 1800s, they were accidentally brought from South America to Europe, Asia, North Africa and western America (Mai, 1977). The pest has attained global distribution through exchange of seed potato and breeding materials (Dalamu *et al.*, 2012). For instance, PCN was detected in a consignment of potato delivered to New York from Peru in 1957 (Mai, 1977). In North Africa, the pest has been reported in Libya, Tunisia, Mali, Morocco (Hlaoua *et al* 2008). In East Africa, PCN has been reported in Kenya, Rwanda and Uganda (Mwangi *et al.*, 2015; Niere and Karuri, 2018). In Kenya, a study on prevalence of the pest was conducted in 20 potato-growing counties after the first

detection showed that 82.8 % of the farms were infested. The pest was especially common in Trans Nzoia, West Pokot and Taita Taveta counties, with a prevalence of about 100 % (Haukeland, 2016).

2.2.2 Life cycle and infection process

Potato cyst nematode has six stages of development: egg, four juvenile stages and adult. Following fertilization and embryogenesis take place in the egg where the first stage juvenile (J1) is formed. Then the J1 moults inside the egg to become juvenile stage two (J2) at this stage the stylet is formed. The stylet helps the J2s to emerge from the egg and the cyst shell ready for invasion. Both male and female J2s respond to the root leachates produced by the hosts. Second stage juveniles enter into the root's vascular tissue of the host crop by inducing a feeding site known as syncytia. Seven days after root invasion the J2 moults into juvenile three (J3) where a sexual dimorphism is formed (Dalamu *et al.*, 2012).

The J3 moults to form the fourth stage juvenile (J4) which is usually flask shaped and measures 0.4 mm in length (Evans and Stone, 2009). Twenty days after entry of the male juveniles into the root, they are attracted by sex pheromones from young mature female juveniles. The female rear end ruptures the root on the surface before fertilization, several males surround the female and numerous matings occur. The male dies shortly after fertilizing the female. Whereas the ovaries of the female rapidly increases in size filling the female juvenile in approximately four weeks, after which fertilization occurs and the cyst is fully formed with embryonated eggs.

2.2.3 Identification and diversity of potato cyst nematode

Until 1920's potato strains of the sugar cyst nematode were considered one species, *Heterodera rostochiensis* (Mwangi *et al.,* 2021). *Heterodera rostochiensis* was not observed as a single species when the population was able overcome certain genes of resistance (Jones 1970). This further showed that *Heterodera rostochiensis* then comprised of two distinguishable species through morphological characteristics. *Heterodera pallida* now known as *Globodera pallida* was identified (Jones (1970). In order to forecast the efficacy and longevity of the existing resistant potato cultivars, it is essential to understand genetic diversity of PCN (Mwangi *et al.,* 2021). Furthermore, it will help in retracing the history of the nematode population thus preventing further spread (Plantard and Porte, 2004).

Potato cyst nematode has co-evolved with solanaceous hosts. It has a limited dispersal in fields which favours inbreeding of juveniles from the same cysts which may affect evolution of the species due to the increase of homozygous (Plantard and Porte, 2004). Cysts nematode therefore require assistance for long distance dispersal such as contaminated farm implements, foot wear, water and infected planting material. If PCN populations are established from a single event, only a few proportions of the original genetic base will be retained (Plantard and Porte, 2004). Multiple introductions on the other hand may result in high genetic diversity.

There are several methods geared towards identification and genetic diversity of PCN. For identification, morphometric key features of cysts and second stage juveniles are commonly used to detect *G. rostochiensis* and *G. pallida* species. *Globodera* cysts are round and smooth, with a tiny projecting neck, no terminal cone, a diameter of 450 µm and brown skin. A single circumfenestration around the vulva slit exists in the perineal area. One of the cyst's key diagnostic features is the perineal region (number of cuticular ridges, distance between fenestra and anus, fenestra diameter, and granek's ratio). Juveniles in the second stage are vermiform annulated and tapering at both ends. Body length, stylet length, tail length, and a hyaline tail part length are key diagnostic features of second stage juveniles (EPPO, 2013). However, these characteristics occasionally overlap with standard diagnostic features in different populations of these two species (EPPO, 2013) making the technique unreliable. The technique must therefore be validated using molecular approaches (EPPO, 2017).

Several polymerase chain reaction (PCR) based techniques have been used successfully to identify the two species (Subbotin *et al.,* 2001; Subbotin *et al.,* 2020). These include use of mitochondria DNA (mtDNA), internal transcribed spacer (ITS) and 28s ribosomal DNA (rDNA) According to Subbotin *et al.* (2001) ribosomal genes are highly conserved to inform species delimitation without being prone to marker saturation. Use of ribosomal genes in multiplex PCR has been successful (Bulman and Marshall, 1997).

Additionally, microsatellite DNA markers also known as simple sequence repeats (SSR) have been used in the genetic diversity studies of PCN populations (Selkoe and Toonen, 2006). The SSR markers are co-dominant and highly polymorphic (Boucher *et al.*, 2013). They are useful in

understanding the phylogenetic history of invasive pests and diseases (Selkoe and Toonen, 2006). The SSR markers have been successfully used to investigate *G. pallida* populations in Europe which revealed that the species might have been introduced from south Peru (Plantard *et al.*, 2008).

2.2.4 Host range

Potato cyst nematode has a narrow host range than the majority of other plant parasitic nematodes (Sullivan *et al.,* 2007; Lilley *et al.*, 2005). With a few exceptions, potato cyst nematode eggs can only hatch in the presence of host plants in the solanaceae family (Mburu *et al.*, 2020). The most common crop hosts of PCN include potato (*Solanum tuberosum, Solanum andigena* and *Solanum phureja*), tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), and night shade (*Solanum nigrum, Solanum vilosum* and *Solanum scubrum*) (Chitambo, 2019). Apart from the above mentioned species, plants in the genera *Lycopersicon, Datura, Physalis, Hyoscyamus, Physoclaina, Salpiglossis and Saracha* are also hosts of the pest (Sullivan *et al.*, 2007).

2.2.5 Spread

Passive spread of PCN is accomplished through movement of infested soil and plant debris by rain water, floods, animals, farm implements, and footwear (EPPO, 2017; Contina *et al.,* 2018). The nematodes can also be disseminated through use of infected and contaminated planting materials. (Mark *et al.*, 2019). The only infective and mobile stage of PCN is the second-stage juvenile, which can move about one meter in search of a host (Wallace, 1968; EPPO, 2004).

2.2.6 Symptoms

Typical PCN infection symptoms are not easily recognised or are not specific as those of bacterial and fungal diseases due to the nature of the infection (EPPO, 2017). The symptoms are related to water stress or nutrient deficiency because nematodes feeding on the roots interfere with water and nutrient uptake (Beeman, 2017). This results in the above ground crop showing stunted growth, yellowing, and wilting leading to formation of small sized tubers and few tubers (EPPO, 2017). Potato cyst nematodes are usually visible with naked eyes on roots (EPPO, 2017).

2.2.7 Influence of site specific factors on prevalence and population density of PCN

There are numerous site-specific factors that contribute to widespread and population build-up of PCN in smaller holder farms. Soil characteristics, cultural practices, human activities, and weather

are examples of such site-specific factors (Avendano *et al.,* 2004). Cultural practices, host crop, soil characteristics, and temperature had a direct impact on spread and population density of plant parasitic nematodes (Dixit, 2019). According to Mogeni (2019) soil characteristics, moisture, temperature, and host plants all had a direct impact on nematode reproduction, survival and infection. The author further reported that soil physical and chemical characteristics influence the prevalence and population dynamics of potato cyst nematodes. For instance, according to Fajardo *et al*. (2011), sandy soils in vineyards had a higher population density of plant parasitic nematodes due to their ease of mobility and infectivity when compared to clay soils. Additionally, studies by Prot and Van Gundy (1981) have shown that clay soils reduced *Meloidogyne incognita* population density. Human activities have been shown to have an impact on the prevalence and population build-up of nematodes (Wachira *et al.,* 2014). Site specific management is therefore important because nematode population densities vary spatially in fields and this assists in designing PCN management strategies to ensure accurate management of PCN.

2.2.8 Yield reduction caused by potato cyst nematode

Potato cyst nematodes suppress yield in potato by forming feeding sites in the vascular tissues which interfere with nutrient and water uptake (Vallejo *et al.,* 2021). The PCN stylet destroys the root cells and injects a degrading enzyme that causes the formation of feeding sites known as syncytia at the vascular tissue (EPPO, 2013). The syncytia act as a metabolic sink which supply all the nutrients required for PCN growth. These nutrients include calcium, glucose, potassium and magnesium and are unavailable to be used in potato tuber filling (Broderick, 2016). However, because of the lack of clear symptoms due to PCN infection, it is difficult to quantify yield loss in relation to symptoms (Vallejo *et al.,* 2021). The yield losses may also be compounded with other diseases pathogen like bacterial and fungi. According to Contina *et al.* (2020) yield losses due to PCN infestation could go up to 80% if left uncontrolled, thus resulting to economic losses in potato production. According to Moreno, *et al.* (1984) yield losses are dependent on the virulence of the pathotype, initial number of cysts or eggs per unit of soil, weather, soil characteristics and farming practices. For instance, a study by Brown (1969) showed that the threshold of PCN in UK is 15 eggs/g soil above which 80 % yield losses can be achieved. A national wide PCN survey carried out in 2016 showed that 82.8 % of potato fields in Kenya were infested by PCN (Mburu *et al.,* 2020). The extrapolation of annual PCN damage to potato in Kenya is estimated to be $127 million

(Mburu *et al.*, 2020). Potato production gaps may widen further due to overreliance of farm saved seed, short or no rotations, lack of information on yield losses and PCN resistant cultivars.

2.3 Management options of potato cyst nematode

In comparison to other pests, managing potato cyst nematodes is more difficult since they live in the soil and the cysts have a hard outer covering that allows them to survive even under unfavorable weather conditions. In potato cyst nematode infested fields, the pest is managed by use of various methods comprising of host resistance, soil amendments, nematicides, cultural practices, bio pesticides and trap crops (Bairwa *et al.,* 2017). None of these methods is however effective by itself (Lopez *et al*., 2013).

2.3.1 Host resistance

Use of resistant varieties is an effective way to reduce PCN population densities in soil (Trudgill *et al.,* 2014). Host resistant varieties are preferred in PCN management over the use of chemicals, which are prohibitively expensive and pose significant humans and environmental risks (Adegbite and Agbaje, 2007). Resistance to a specific pathotype of PCN has been provided by R-gene resistant cultivars (Bakker *et al.*, 2006). On eight linkage groups in potato, 14 PCN resistance gene loci have been identified (Tomczak *et al.*, 2011). The commonly used resistance genes in *Globodera rostochiensis* include *Grol2, Grol3*, and *Grol4*. These confer partial resistance whereas *H1, Grol* and *GroIV* give near absolute resistance (Faggian *et al.*, 2012). Potato varieties having the *H1* gene have been developed. The *H1* allele in them hinders the development of specialized feeding structures, syncytia, thus causing juveniles to starve and die whereas Ro1 strain of PCN cannot survive on resistant cultivars. Some male juveniles survive however, the development cycle is stopped since the female is absent for further development (Blok *et al.*,2008). Cultivars which are resistant to the Ro1 strain of *Globodera rostochiensis* have been shown to reduce cyst density in soil by 80-90% after each crop and increase yield over a season (Blok *et al.,* 1997). However, the lack of host resistance varieties usually limits this mode of control (Blok *et al.,* 2008; SASA, 2010).

2.3.2 Cultural practices

Use of agricultural practices such as organic amendment, intercropping, washing of farm implements, machinery and foot wear, use of certified seed by farmers, and removal of volunteer

host crop have been tested and demonstrated to be effective in lowering PCN density (SASA, 2010; Lopez *et al.*, 2013;.Sumner *et al.*, 2002). To avoid further PCN infestation, farmers should routinely plant uninfected seed, disinfect farm tools, and footwear and practice crop rotation. These are safe and effective nematode management practices that farmers can easily implement.

2.3.3 Crop rotation and cropping sequence

Crop rotation is a cropping system where different types of crops are grown in the same area sequentially, this technique has shown to be effective in PCN management (Siddiqui *et al.*, 2019). Farmers generally accept the practice because it improves soil fertility, crop productivity in addition to reducing crop losses attributed to pests. It has been demonstrated that alternating a non-host crop, velvet bean, with soya (a host crop), lessens the population density of *Meloidogyne incognita* juveniles due to starvation (Widmer *et al.*, 2002). Previous research has shown that a six year crop rotation without a host is the most effective way of suppressing the PCN density, as there is substantial reduction of viable eggs (SASA, 2010). However, in Kenya potato production systems are characterised by mono-cropping, relay cropping with minimal crop diversity, and continuous cropping (only a few farmers can rotate for the recommended 6 years due to limited land sizes). This has a number of consequences for productivity and profitability including deteriorating soil quality and high risk for soil-borne diseases and pests (Muthoni *et al*., 2013). According to Haukeland (2016) a short rotation or no fallow periods in potato production systems in Kenya exacerbates the cyst population density.

2.3.4 Chemical control

Nematicides are an effective method of nematode management (Khalil *et al.,* 2012). Initially nematicides in the market were used as sterilants or fumigants such as methyl bromide, insecticides (oxamyl, ethoprop) and fungicides (Desaeger, 2021). They are either volatile or non-volatile and consist of halogenated carbons which produce methyl isothiocyanate (methan sodium) (Whitehead, 1986). The volatile nematicides are broad spectrum and are generally effective against cysts, juveniles, eggs and other pests and diseases. Non-volatile nematicides include carbamates and organophosphates. Nematicides are usually drenched into the soil at planting and are supplied in liquid or in granular form (Ngundo, 2016). For instance, oxamyl and fluopyram (carbamates) nematicides have successfully been used against potato cyst nematodes on potato and have been reported to prevent nematodes from finding the host by releasing nerve toxins (Hedfi *et al.*, 2017;

Desaeger and Zasada, 2021). However, they are expensive, toxic, and require careful handling and specialized equipment for application (Chitambo, 2019). There are growing concerns about the negative effects of nematicides since they remain in ground water. Besides, the high residue levels left in tubers they pose a risk to the environment, humans and animals thereby discouraging their use (Lopez *et al.,* 2013). Synthetic nematicides result in killing of the beneficial micro-organisms thereby causing an imbalance in the ecosystem (Ajitomi *et al.,* 2018). For example, metham sodium was reported as being effective in suppressing PCN but it reduces the beneficial microbial communities, mineralisation of nitrogen and carbon in the soil (Aires *et al.,* 2009).

2.3.5 Biological control

Biological control agents parasitize nematodes through different mechanisms which include (1) production of destructive metabolites such as hydrolytic enzymes (chitinase) and mycoparasitism using antibiotics, (2) root colonization, (3) plant growth promoters, and (4) the induction of resistance of host plants (Samaa *et al.,* 2010). Biological controls that have shown success in controlling plant parasitic nematodes include yeasts, nematophagus fungi, and antagonistic bacteria (Mokbel and Alharbi, 2014). Isolates of *Bacillus subtilis* and *B. thuringiensis* have been reported to produce an antibiotic bacteriocin which have shown efficacy in controlling *M. incognita* (Samaa *et al.*, 2010). Nematophagus fungi have been studied extensively and have been observed to parasitize the vermiform nematodes and egg masses of nematodes by trapping them using a special hyphae to capture the nematodes (Mokbel and Alharbi, 2014). *Saccharomyces cerevisiae* is a growth promoter yeast used in different crops and has shown a suppressive effect on root knot nematodes (RKN) under greenhouse conditions. *Paecilomyces lilacinus* a fungus that is in Kenyan market has been used against *G. rostochiensis* and has shown suppressive effects of 68 % on egg hatch (Lopez *et al.,* 2013).

2.3.6 Green manure

Allelochemicals produced by some green manures have been shown to be useful for controlling soil borne pests and diseases (Lazzeri *et al.,* 2004). For example plants belonging to *Brassicaceae* family produce biologically active compounds that are nematicidal (Oliviero *et al*., 2018; McGuire, 2003) The effective isothiocyanates are 2-phenylethyl- isothiocyanate and methyl which are reported to destroy or suppress soil borne diseases, nematodes and weeds (Aires *et al.,* 2009). For instance, *Brassica rapa*, collard, cauliflower and kale showed suppressive effects on soil

potato cyst nematode (Aires *et al*., 2009). Also, sorghum, Sudan grass, green manure showed a similar mechanism in combating root knot nematode infestations (RKN) (Widmer *et al.*, 2002).

2.3.7 Botanical pesticides

Plant extracts provide secondary metabolites and promote biological activity against nematodes. In the farm, botanical pesticide are not persistent since they are broken down to into less dangerous substances. Therefore, no harmful residues are deposited into the environment (Taniwiryono *et al.*, 2009). Botanical extracts have secondary metabolites, which serve as potential nematicides. These products include alkaloids, fatty acid, phenols, polyacetylenes, sesquiterpenes, glucosinolates thienyls, isothiocyanates and diterpenes. For instance, it has been demonstrated that mortality of root knot nematode occurred when exposed to crude plant extracts from *Tagetes erecta* and medicinal plants extracts (*Calendula officinalis, Amborosia maritima, Origanum vulgare*) (Mokrini *et al.*, 2017). Additionally, 100 % juvenile mortality and egg hatch inhibition of *Meloidogyne incognita* occurred when exposed to plant crude extracts from *Coccinia grandis, Achyranthes aspera, Solanum xanthocarpum, and Ageratum conyzoides* (Khan *et al.*, 2019).

2.3.8 Trap crops

Trap crops could be used as a non-chemical control method for PCN management. These crops cause PCN to hatch and infest the plant but prevent the pest from completing its life cycle (Timmermans, 2005). In UK and South America, *Solanum sisymbriifolium* is commercial PCN trap crop and has been reported to reduce PCN significantly from within a range of 65 %-75 % (Clayton *et al.*, 2008; Scholte, 2000; Timmermans, 2005). Commercial adoption of this trap crop is very limited due to the loss of income generating crop in the trap crop year (Clayton *et al.*, 2008). *Solanum scabrum* and *Solunum villosum* (night shade) has shown potential in controlling potato cyst nematodes with different accessions showing different responses (Chitambo.,2019). Unlike other *Solanaceace* crops, night shade shows lower risks to *Phytophthora infestans* (Sparkes, 2013). Potato cyst nematode trap crops have been one of the promising crop rotations and they are grown before a potato crop to reduce the PCN density (Clayton *et al.*, 2008). They allow the cysts to hatch but don't allow further development since the second stage juvenile development is restricted due to necrotic degeneration of synctial feeding cells (Chitambo, 2019).

CHAPTER 3: DIVERSITY OF POTATO CYST NEMATODE POPULATIONS IN NYANDARUA, MERU AND NAKURU COUNTIES BASED ON MORPHOMETRIC AND SIMPLE SEQUENCE REPEAT MARKERS

3.1 Abstract

Potato cyst nematodes (*Globodera* spp) are common in many of Kenya's potato-growing counties, but little is known about their genetic diversity. The aim of this study was to identify and determine their genetic diversity of potato cyst nematode (PCN) populations present in three potato growing counties namely Nyandarua, Nakuru and Meru in Kenya. Both morphometric and molecular analyses were used to identify the PCN species present from 88 PCN samples collected from the three Counties. Twenty simple sequence repeats markers, four of which were newly designed for this study were used to genotype the 88 PCN samples. Morphometric analyses were inconclusive in differentiating between *Globodera pallida* and *Globodera rostochiensis*. The polymerase chain reaction (PCR) amplicons were observed at 434 base pairs amplified with primer (ITS5/PITSr3) specific for *G. rostochiensis* in all the PCN samples. Screening of the 20 SSR markers resulted in identification of four polymorphic markers specific for PCN. The expected heterozygosity (*H*e) and Shannon's index (*I*) ranged from 0.320 to 0.500 and from 0.500 to 0.693, respectively, across the three populations. As a result the *H*e and *I* values indicate a limited range of genetic diversity and heterozygote deficiency between the samples. The markers' mean polymorphism information content (PIC) ranged from 0.344 to 0.365 indicating an intermediate polymorphism (PIC $\geq$ 25) across the populations. Furthermore, analysis of molecular variance (AMOVA) revealed that the highest genetic variance 96% ($P<0.001$) came from within the population. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phylogenetic tree was constructed Based on Nei's genetic dissimilarity and the entire population was grouped into two major clusters and five sub-clusters. Principal coordinate analysis (PCoA) revealed that the variance accounted for by the first three principal coordinates were 27.73, 28.89 and 24.42 respectively. The Bayesian model-based population structure analysis assembled the populations into 6 (K=6) distinct genetic structures based on the highest ΔK value (ΔK=8.25). The fixation index (Fst) ranged from 0.021 to 0.048, indicating that the three *G. rostochiensis* populations had low genetic differentiation. Overall, the findings demonstrated limited gene flow across the regions.

3.2 Introduction

Population genetics has been used to investigate pest evolution, host-parasite interactions, and the development of effective and efficient control methods (Gautier *et al.*, 2021). A pest's genetic diversity is determined by the species, dispersal abilities, population size, and mode of reproduction (Alenda *et al.*, 2014). These traits aid in the pest populations' genetic diversity as well as their ability to adapt to different environmental conditions and management methods (McDonald and Linde, 2002). Genetic diversity of plant parasitic nematode populations from various geographical areas may thus aid in predicting the efficacy and durability of some management strategies. Plant breeders and plant pathologists have also used species diversity to better understand pathogens and develop new varieties (Boucher *et al.*, 2013). Due to the economic significance of PCN in Kenya, identifying and determining genetic diversity is critical for developing appropriate management strategies.

Morphometric and molecular methods were used to identify cyst nematodes such as potato cyst nematodes (Mwangi *et al.*, 2021), the tobacco cyst nematode, *G. tabacum* (Alenda *et al.*, 2014) and the cereal cyst nematode *Heterodera Avenae* (Wang *et al.*, 2017). The two approaches morphometric and molecular are important in the complementary diagnostic roles of identification of PCN species (Den Nijs and Karssen, 2004). Morphometric analysis of the perineal area of cysts and J2s is critical for differential identification of the two PCN species (Djebroune *et al.*, 2021). However, sometimes the morphometric features overlap and it becomes difficult to distinguish the two species. Therefore, there is need to explore deoxyribonucleic acid (DNA) based techniques which are more accurate in differentiating members of the two species. The validated molecular assays for *Globodera* identification are based on amplification of the ribosomal internal transcribed spacer region (ITS), or ITS ribosomal DNA (ITS rDNA) (Skantar *et al.*, 2011).

To examine genetic diversity and monitor the spread of plant parasitic nematodes, researchers have utilized ITS rDNA and simple sequence repeats (SSRs) or microsatellites (Boucher *et al.*, 2013). The SSR markers are one of the reliable tools for studying population genetic diversity studies (Handayani *et al.*, 2020). Microsatellite markers are short tandem DNA repeats with 2-8 motif (Handayani *et al.*, 2020). The SSR markers are highly reproducible, codominant, abundant and evenly distributed in the nematode genome, they are either coding or noncoding regions (Omondi

et al., 2016). Moreover, SSR markers were shown to be highly transferable between species or closely related genera, allowing discrimination between closely related species in nematodes (Bornet *et al.*, 2002; Kostova, 2021). Earlier study in Europe has demonstrated the successful use of microsatellite markers to examine the genetic diversity of *Globodera pallida* populations (Plantard *et al.*, 2008). In Peru, SSRs were used to shed light on the phylogeographical structure and the allelic diversity of *G. pallida* populations (Picard *et al.*, 2004). Boucher *et al.* (2013) employed microsatellite markers to assess the genetic diversity and phylo-geographical history of both *G. pallida* and *G. rostochiensis* populations in Canada.

Genetic diversity of PCN will aid in generating essential information in better understating of the pest and will go a long way towards development of PCN tolerant potato varieties and in devising strategies of protecting a crop. Hence, the present study aimed at identifying the PCN species present in Kenya and assessing the genetic diversity among the genus *Globodera* from PCN populations collected from three potato growing counties namely Meru, Nakuru and Nyandarua using SSR markers.

3.3 Materials and methods

3.3.1 Source of potato cyst nematode populations

The PCN populations used in this study were obtained from three potato-producing Counties in Kenya: Nyandarua County (00º 55` N and 036º 62` E), Meru County (00º 35 N and 037º 63`E), Nakuru County (00º 32` E and 36º 48` 04`N).

3.3.2 Soil sampling

Soil samples were taken from three agro-ecological zones (AEZs) in each county: Upper highland 3 (UH3), Upper highland 2 (UH2) and Lower highland 4 (LH4). Ten farms were chosen at random from each of the three AEZs in each county. Ninety (90) soil samples were collected in total. Each farm had a random sampling of 10 sub-samples collected from the potato rhizosphere at a depth of 30 cm. Each sub-sample weighed about 40 g and were combined in Khaki bags (size 1kg) to form a composite sample of about 400 g transported in cooler boxes and kept at ambient temperature 20–25 °C.

3.3.3 Cyst extraction

The composite soil sample was thoroughly mixed and 300 g of soil taken, cyst were extracted from each composite sample using the Fenwick Can method (Fenwick, 1940). The cysts were isolated under a binocular microscope (EPPO, 2013). Three cysts containing eggs and juveniles were picked from each of the samples for morphometric and molecular analysis.

3.3.4 Morphometric identification

Morphometric identification was conducted at International Centre of Insect Physiology and Ecology (*icipe*), in Nematology Laboratory**.**

Fixation and permanent preparation of perineal region of cysts of *Globodera* spp

For identification of *Globodera* spp., the perineal region of cysts were examined. Three cysts from each sample were pre-soaked in 2-3 drops of water on a slide for five minutes and cut with a surgical blade. The cysts' perineal region was then immersed in glycerine on a slide, then covered with a cover slip and sealed with nail polish (Hooper, 1986).

Fixation and permanent preparation of second stage juveniles of *Globodera* spp.

Second-stage juveniles were picked and put in 1.5 ml Eppendorf tubes with distilled water until needed for morphometric and molecular analysis. Three second-stage juveniles obtained from each of the three cysts kept in Eppendorf tubes, were then picked and put in a cavity glass block. Excess water was removed using a pipette under a dissecting microscope. Tri-ethylamine formalin (TAF) fixative was added into the cavity glass block containing the three J2s. After immersing the juveniles in fixative, the cavity glass block was placed in a water bath at 60^{o}C for 5 minutes. The nematodes were incubated in the fixative overnight at room temperature in a desiccator. The cavity glass block containing the fixed J2s was then placed in a jar having absolute ethanol. The nematodes were then immersed in glycerin on a slide, covered with a cover slip and sealed with nail polish (Hooper, 1986).

Morphometric measurements of perineal region of cysts and second stage juveniles

The specimens were examined under a stereo microscope (Leica DM 1000 LED) linked to a digital microscope camera (Leica DFC 295). The morphometrics of the perineal region cyst s and second stage juveniles were taken using taxonomic keys of identifying *Globodera spp* as described by EPPO (2013). The specimens were measured using Leica Application Suite software Version 3.6.0

(Bacici *et al.*, 2013).The anus to fenestral edge distance, fenestral diameter, number of cuticular ridges and granek's ratio were used to identify the perineal region of the cyst. The body, tail, hyaline and stylet length were measured, while the second stage juveniles stylet knob shape was noted (EPPO, 2013).

3.3.5 Molecular identification of the species

Deoxyribonucleic acid (DNA) extraction

Following the manufacturer's instructions, genomic DNA was extracted using the ISOLATE II Genomic DNA kit (Bioline). Two J2s were handpicked individually under a stereoscope microscope from the cysts used during morphometric characterization and put in 1.5 ml Eppendorf tubes in which 20µl10X lysis buffer was added. The nematodes were ground with a pestle and 5 µL of Proteinase K (20 µg μL^{-1}) was added to each tube. They were incubated for 1 hour at 60 °C and 10 minutes at 94°C before being briefly cooled on ice and centrifuged to remove debris. The supernatant was collected and kept at −20°C until needed.

Quality determination of DNA

Genomic DNA was visualized in 1.5 % agarose gels using the gel electrophoresis method based on a modified protocol of Lee *et al.* (2012). Tris Acetate Ethylenediaminetetraacetic acid (EDTA) (TAE) (1% w/v) buffer 50 ml was added to a clean flask, followed by 0.75 g of agarose. The mixture was then microwaved until the agarose dissolved completely (approximately 3 min). The hot agarose mixture was allowed to cool under running water to about 50 ^{o}C before adding 5 µl of 1% GelRed. The warm solution was then poured into the gel tray in which a comb was inserted to form slots. For 30 minutes, the gel was allowed to solidify; the comb was carefully removed to avoid pulling out the bottom of each well which can cause the samples to leak out. After that, the gel was then placed in a buffer tank and covered with a (1-2 mm) buffer. Thereafter, 6 µl of loading solution containing 5 µl DNA sample, 2 µl loading dye (bromophenol blue) was loaded to the wells of the gel. BIOLINE hyper ladder 100 bp was used and was run in parallel with the test samples. The gel was run at100V, 100W for 40 minutes and using a gel documentation system the gel was visualized under UV light and photographed. The quantity and quality of DNA were assessed by visual comparison of the sharpness and intensities of the DNA bands.

Polymerase Chain Reaction (PCR)

As described by EPPO (2017), each of the 88 samples was subjected to a modified multiplex polymerase chain reaction (PCR) protocol to distinguish the species (*G. pallida* and G. *rostochiensis*) present in soil samples. The ribosomal internal transcribed spacer (ITS) region was used in the multiplex PCR reactions (White, 1990). The PITSr3 (5' AGCGCAGACATGCCGCAA-3') primer specific for detection of only *G. rostochiensis* and PITSp4 (5'-ACAACAGCAATCGTCGAG-3') primer specific to detect only *G. pallida* in combination with the forward universal primer ITS5-(5' CGTAACAAGGTAGCTGTAG-3') were amplified (Bulman and Marshall, 1997;White, 1990). Each PCR reaction contained a total of 25 µl reaction volume, having 0.5 µl Taq polymerase, 8 µl 50X DNA reaction buffer, 0.5 µl of each primer (forward and reverse) and 8 µl of DNA template 6.5 µl nuclease free water. The reactions were carried out in an Applied Biosystems ProFlex thermocycler that was programmed. The PCR cycling conditions were: denaturation of double strand DNA at 94°C (3min.) 94°C (30 sec), primer annealing at 55°C (30 sec) 40 cycles of polymerization at 72°C for 30 sec each, followed by 5 min at 72°C.

Agarose gel electrophoresis

To analyze each of the 88 samples, 10 µl of PCR products of each sample was mixed with 0.5 µl DNA loading buffer and was separated using 1.5 % agarose stained with 1X GelRed. A standard DNA marker 100bp (Biolabs) was also run on each gel. Electrophoresis was performed in 1 x TAE buffer at 100V, 100W for 40 minutes. The Gel Doc EZ system was used to visualize the gel under ultra violet light. A 100bp ladder was used to estimate the sizes of the successful amplicons. Samples yielding a PCR product of 265 bp amplicon were considered *G. pallida* whereas samples giving a PCR product of 434 bp amplicon were considered *G. rostochiensis* (Bulman and Marshall, 1997). Samples that produced no PCR product or produced a PCR product beyond these ranges were thought to belong to species other than *G. pallida* and *G. rostochiensis*.

3.3.6 Genetic diversity of PCN using SSR markers

Microsatellites development and screening for polymorphism

Twenty SSR markers were used to evaluate the genetic diversity of 88 PCN samples. Sixteen SSR markers (Gr50, Gr67, Gr75, Gr85, Gr90, Gr91, Gr96, Gr82, Gr70, Gr79, Gr94, Gp109, Gp116, Gp118, Gp126, and GP135) were chosen from previous research based on their high polymorphic information content (PIC) (Boucher, *et al*., 2013) (Table 3.1). Four new primers, GRM1 and GRM2 specific for *G. rostochiensis*, GPM3 and GPM1 specific for *G. pallida* both forward and reverse were designed for this study (Table 3.1). To design these markers, *G. rostochiensis* and *G. pallida* genome were downloaded from Genebank (www.ncbi.nlm.nih.gov/dbEST). SSR locator software (http://microsatellite.org/ssr.php) was then used to select the SSR markers. Then primer 5.0 software (www.premierbiosoft.com) was used to design the microsatellite marker using two criteria: primer size 18-20 oligonucleootide length below 250 bp and melting temperature between 50 °C – 63 °C (optimum 60 °C). The SSR markers were synthesized by Macrogen (South Korea). Table 3.1 shows the sequences of the SSR primers used in the PCR reactions. Thirteen of them were specific for *G. rostochiensis* and 7 of them belong to *G. pallida*. All the 20 SSR markers were screened for polymorphism using four random samples from each county. Primers with polymorphism were selected for large scale PCR amplification and agarose gel electrophoresis using 88 PCN populations.

Table 3.1. Characteristic of twenty simple sequence repeat (SSR) loci of *Globodera rostochiensis* and *Globodera pallida*, including forward and reverse primer and annealing temperature

No	Primer ID	Species	Primer forward sequence (5´–3´)	Primer reverse sequence (5´–3´)	Motif	Size (bp)	Annealing Temp° C (Ta)	Polymorphic Markers
1	GRM1	*G. rostochiensis*	TTCCTTCAGACTTGTTCAGAG	GAGAGAGTGAGGAGAGAAAGC	(CT)6	155	55	No
2	GRM2	*G. rostochiensis*	TTTAATAGCTTGGCTCACAAC	ATCTGCCAAACACACAGAG	(TA)5	205	51	Yes
3	Gr50	*G. rostochiensis*	TTTGTCGGGCCTAAAAAGTG	CAAGTTCCTCTCCTATGCCG	(ACCA)5	322	55	No
4	Gr67	*G. rostochiensis*	ACCTGAACGTCGTCATTTCC	TTTTCTTACCCGAATGGCAC	(GT)5	180	52	Yes
5	Gr75	*G. rostochiensis*	CCAAAAATGGCACATCCACT	GCTGCTGTCGTACGCAAGAT	(CA)10	147	55	No
6	Gr85	*G. rostochiensis*	CCAAAAATTGATTGGCATCC	AATATCGCGTTGTTCCCAAG	(CA)7	123	55	No
7	Gr90	*G. rostochiensis*	CGTAGTACGACGCGTTCAAG	GATCCGGCACTGGAGTACAT	(AC)12	115	55	Yes
8	Gr91	*G. rostochiensis*	GGTTGAAATGACGCAAGTGA	GATCTAATCCTTCCGGGCTC	(GT)5	112	55	No
9	Gr96	*G. rostochiensis*	CGGATAAAAAGCTTAAAACCCT	TGCAAAACGTTCAGATATTTT	(AC)8	103	54	No
10	Gr82	*G. rostochiensis*	CGTCTGCATTTTGTCGTGTT	GTTCCGGCCAAATCCGTC	(GA)5	131	55	No
11	Gr70	*G. rostochiensis*	AACACAAAATGCGAAAGCG	TTTTTCTTATTGCTTCCCTTCC	(AG)7	169	54	No
12	Gr79	*G. rostochiensis*	ATTATCCCCCAAAAGTGGCT	TCGATTAAAAGGCATTTGGC	(AC)5	136	51	Yes
13	Gr94	*G. rostochiensis*	GTACATCAATTGACACCTGC	GAGTAAAGACATAAACTAGAGTGAT	(TC)7	107	54	No
14	GPM1	*G. pallida*	AAGGAAAGCATCCCTAAAGT	GATTAGAATGCACCAAATGAC	(GC)5	333	54	No
15	GPM2	*G. pallida*	TTGACCCTGTATTCTTACTGTTC	CATTCGGATAAACATCTCTGA	(TG)4	289	53	No
16	Gp109	*G. pallida*	TCTCGCAGAAGGGAAAAGAA	TAAAAGACGGAAGAACGGGA	(ACGG)5	133	55	No
17	Gp116	*G. pallida*	ATTCATTCGCAATGTTTCCC	TGGAAATGTGAGAAAGGGCT	(CGTC)4	141	54	No
18	Gp118	*G. pallida*	ACCGATGAAGAACATCGTCC	TCGTTCCGTCTTCGTAATCC	(TCCG)4	133	52	No
19	Gp126	*G. pallida*	GTTATTGTGGCGGATGGAAT	GTACTGTATGATGCCGGGCT	(GATT)4	192	55	No
20	GP135	*G. pallida*	GCGAAATGAACGGTCGTAGT	ATTACATTGCCCAAATCGGA	(GA)7	146	54	No

Selected or not selected for PCR amplification of all 88 PCN population, bp; base pairs

Polymerase Chain Reaction (PCR)

The PCR reactions were carried out in a 25µl reaction volume; 0.5 µl Taq polymerase, 8 µl 50X DNA reaction buffer, 0.5 µl of SSR primer pair and 8 µl of DNA template and 6.5 µl nuclease free water. Each test included controls without nematodes. The reactions were performed in a programmable thermocycler under the following conditions: 94 ^{o}C for 3 min, followed by 40 cycles of denaturation at 94 ^{o}C for 30 sec each, annealing (temperature of each SSR primer is indicated as Ta in table 3.1 for 30 sec) and extension at 72 ^{o}C for 30 sec and with a final elongation at 72 °C for 5 min, followed by a final extension at 60 ^{o}C for 30 min. To validate the 20 SSR markers, screening was carried out using agarose gel electrophoresis using four samples from each county.

Agarose gel electrophoresis

To analyze each of the 88 samples, 10 µl of PCR product of each sample was mixed with 0.5 µl DNA loading buffer and was separated using 1.5 % agarose stained with 1X Gel red prepared according to Lee *et al*.(2012). A standard DNA marker 100bp (Biolabs) was also run on each gel. Electrophoresis was performed in 1 x TAE buffer at 100V, 100W for 40 minutes. Using the Gel Doc EZ system, the gel was visualized under ultra violet light (Bio-Rad). For all the 88 samples, the SSR amplified bands were scored as 1/0 (presence/absence)

3.4 Data analysis

Statistical analysis of morphometric characteristics of juvenile and cyst parameters was done using Microsoft office Excel software. The mean range of the characteristics were then compared with standard measurements as recorded in EPPO, (2013).

For each PCN sample, the SSR amplified bands were scored as 1/0 (presence/absence). Only bands that were reproducible and polymorphic were included in the study. Based on the alleles identified in the samples, genetic diversity parameters were computed using GenAlEx version 6.5 (Banks and Peakall, 2012). The polymorphic information content (PIC) and allele frequency were calculated using power maker software version 3.25. The PIC was calculated using Nei's statistic (Nei, 1973): $PIC=1-\sum(pi2)$,

Where pi is the frequency of the ith pattern for microsatellite marker i and is summed across n patterns.

Analysis of molecular variance analysis (AMOVA) was computed using GenAlEx software version 6.5. The principal coordinate analysis (PCoA) was utilized to provide graphical representation of genetic relationship of the PCN population studied using GenAlEx version 6.5. Dendrograms were generated using an unweighted pair group method of cluster analysis and arithmetic averages (UPGMA) based on the matrix of Nei's genetic distance with POPGENE version. 1.31. Genetic distances were calculated according to (Nei and Li, 1979) based on the likelihood that the amplified fragment from one genotype will be present in another genotype. The Bayesian model clustering analysis was used to estimate the most likely number of groups/populations (K) by STRUCTURE version 2.3.2 software, this model allowed admixture of populations (Pritchard and Stephens, 2000). The results were then imported to STRUCTURE HARVESTER software to test the proper *K* by using *Inp* value (Earl, 2012).

3.5 Results

3.5.1 Population identification using morphometric and molecular techniques

Morphometric identification of PCN species

Two of the 90 samples collected did not have cysts. All extracted cysts were globular in shape, golden brown in colour and had a neck protruding that helps the cyst to attach to the roots. The cysts perineal region of the cysts had two openings of varying sizes, the larger being the vulva and smaller being anus with a V shape. Between the two openings (vulva and anus) were cuticular ridges (Figure 3.1 a). The second stage juveniles were vermiform in shape (Figure 3.1 b) with stylets having round basal knobs (Figure 3.1 c) and hyaline part and true tail (Figure 3.1 d).

Morphometric analysis of J2s and perineal region of cyst of the 88 PCN samples collected from the three potato growing counties showed overlap of the standard measurements (EPPO, 2013) of the two species.

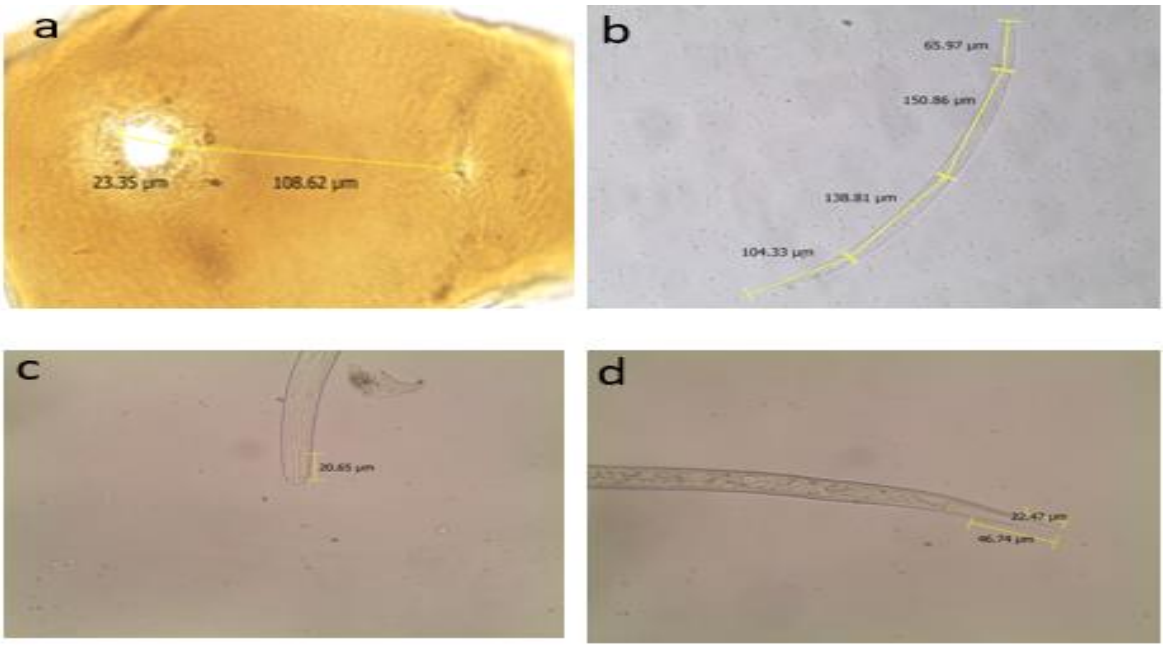

Figure 3.1 PCN morphometric measurements a) perineal region, b) second stage juvenile, c) stylet and d) juvenile tail at a 40× objective on a compound microscope

The mean values and the range of cyst cones morphometric characteristics differed slightly between the three regions of Nyandarua, Nakuru and Meru. For instance, the distance between fenestra and anus was 31.8-94.6, 38.8-77.7 and 35.2-93.0 µm for populations from Nyandarua, Nakuru and Meru respectively. The respective fenestra diameters of the perineal region of the cysts were 14.3-28.1, 13.6-26.2 and 14.3-28.9 µm in Nyandarua, Nakuru, Meru county respectively. Granek ratios for PCN samples from Nyandarua, Nakuru and Meru were 1.8-5.6, 2.2-4.2 and 1.9-5.4 µm respectively (Table 3.2).

There were minimal variations of all morphometric values of the characteristics obtained from second stage juveniles (J2s) of PCN samples from Nyandarua, Nakuru and Meru. The morphometric measurements of second stage juveniles from the three potato regions are shown in Table 3.3. The PCN samples exhibited a body mean length ranging from 434.7- 451 µm which according to EPPO (2013) classification, overlapped with the body length values of *G. rostochiensis* and *G. pallida*. The J2s stylet lengths ranged from 21.6-22.1 µm (Table 3.3) for all the specimens, which were within the values reported by EPPO (2013) for *G. rostochiensis* (Table 3.4). The stylets had rounded basal knobs.

Table 3.2. Morphometric characteristics of cysts from Meru, Nyandarua and Nakuru populations

County	AEZ	No. of Samples	Cysts			
			Distance from Fenestra to anus (μm)	Fenestra diameter (μm)	Graneks's ratio	Number of cuticular ridges
Nyandarua	LH4	10	54.6± 2.56 (31.8-94.6)	19.4±0.51 (14.4-24.7)	2.8±0.12 (1.8-4.0)	14.0±0.49 (9.0-19.0)
	UH2	10	54.9± 2.72 (32.9-89.9)	18.9±0.52 (14.3-28.1)	2.9± 0.11 (1.9-4.2)	14.9 ± 0.52 (10.0-21.0)
	UH3	10	58.9±3.20 (35.9-77.7)	19.1±0.43 (14.8-22.9)	3.1±0.14 (2.1-5.6)	15.9±0.58 (11.0-25.0)
	SE		15.53	2.66	0.71	2.99
	SD		1.63	0.28	0.08	0.32
Nakuru	LH4	8	51.1±1.33 (41.1-67.1)	17.3±0.39 (13.6-21.4)	3.1±0.09 (2.2-4.2)	14.8±0.32 (12.0-17.0)
	UH2	10	52.7±0.55 (38.8-76.4)	19.5±1.72(15.4-26.2)	2.7±).07 (2.1-4.1)	15.9± 0.53 (11.0-22.0)
	UH3	10	53.6±1.75 (43.9-77.7)	18.6+0.39 (15.6-23.8)	2.9± 0.08 (2.1-3.9)	16.2± 0.43 (12.0-21.0)
	SE		8.6	2.57	0.42	2.42
	SD		0.93	0.28	0.05	0.26
Meru	LH4	10	63.5± 3.17 (50.0-80.4)	20.7± 0.62 (14.3-27.9)	3.1± 0.13 (2.0-5.4)	16.2± 0.60 (11.0-25.0)
	UH2	10	65.7±3.75 (35.2-78.8)	21.1±0.58 (14.5-28.3)	3.2± 0.16 (2.3-3.6)	15.9±0.71 (11.0-26.0)
	UH3	10	61.2± 2.31 (40.9-93.0)	20.1± 0.53 (15.5-26.1)	3.1± 0.11 (1.9-4.9)	15.4± 0.55 (11.0-22.0)
	SE		8.57	2.57	0.42	2.42
	SD		0.93	0.28	0.05	0.26

Measurements represented as mean ± standard error (s. e.), range data is given in parenthesis, AEZS: Agro-ecological zones, LH4: Lower highland 4, UH2: Upper highland 2, UH3: Upper highland 3, SD: Standard deviation, SE: Standard error

Table 3.3. Morphometric characteristics of second stage juveniles from Meru, Nyandarua and Nakuru populations

County	AEZ	No. of Samples	Second-stage juveniles			
			Body length	Tail length	Hyaline region	Stylet length
Nyandarua	LH	10	444.6 ±3.5 (372.0-516.6)	49.2±0.04 (40.3±59.6)	24.8±0.28 (19.5±33.3)	22.1±0.09 (20.0-23.9)
	UH2	10	439.6±3.01 (349-497.16)	48.8±0.21 (44.8-53.2)	24.2±0.25 (19.3+29.6)	21.6±0.09 (19.6-23.3)
	UH3	10	451.2±3.11 (370.7-511.5)	49.1±0.30 (41.8-56.7)	24.4±0.23 (19.2-29.6)	21.8±0.09 (19.5-24.2)
	SE		31.09	2.76	2.40	0.88
	SD		1.89	0.17	0.15	0.05
Nakuru	LH	8	447.7±4.33(380.7=525.1)	48.7±0.69 (24.6-56.4)	27.2±0.62 (20.2-46.7)	21.6±0.14 (20.2+24.1)
	UH2	10	445.2±1.92 (396.8-483.1)	49.2±0.22 (44.6-55.5)	26.2±0.32 (19.2-34.9)	21.8±0.09 (19.9-23.9)
	UH3	10	434.7±3.00 (370.2-486.1)	48.2±0.27 (41.5-53.3)	24.4±0.26 (17.1-30.9)	21.6±0.10 (19.8-23.9)
	SE		24.29	2.94	3.27	0.91
	SD		1.68	0.20	0.23	0.06
Meru	LH	10	446.2±2.08 (388.2-482.7)	49.7±0.24 (44.9-54.7)	24.7±0.29 (18.4-34.0)	21.7±0.08 (19.0-23.2)
	UH2	10	438.6±3.01 (332.8-501.4)	49.2±0.30 (44.0-55.8)	25.1±0.27(20.3-33.7)	21.7±0.10 (19.3-23.7)
	UH3	10	448.6±1.99 (399.9-489.8)	49.5±0.23 (44.3-53.7)	24.5-0.27 (19.2-32.9)	21.6±0.09 (19.5-23.7)
	SE		22.1	2.36	2.6	0.8
	SD		1.4	0.15	0.15	0.1

Measurements in μm and represented as mean ± s.e., range data is given in parenthesis; AEZS: Agro-ecological zones, LH4: Lower highighland 4, UH2: Upper highland 2, UH3: Upper highland 3, SD: Standard deviation, SE: Standard error

Table 3.4. Morphometric characteristics of cyst cones and second stage juvenile characteristics as given in OEPP/EPPO Bulletin, (2013)

Species	Second-stage juveniles				Cysts			
	Body length	Tail length	Hyaline region	Stylet length	Distance from fenestra to anus	Fenestra diameter	Graneks's ratio	No. cuticular ridges
G. pallida (EPPO 2013)	452- 486	50-53	26-27	23-24	48-54	**	2.1-2.5	12-17
G. rostochiensis (EPPO 2013)	392-468	44-51	20-27	20-22	51-70	**	3.0-4.5	17-20

Measurements in μm, EPPO; European and Mediterranean Plant Protection Organization

DNA quantity and quality

Based on the agarose gel electrophoresis, the quantity and quality of DNA for most samples were good as shown in Figure 3.2.

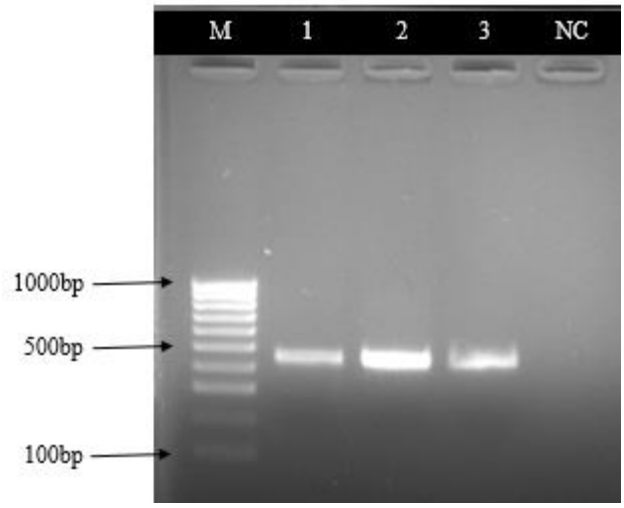

Figure 3.2. Agarose gel electrophoresis image showing the quality of potato cyst nematode DNA extracted
(Lane M-100 bp molecular marker, lane 1- a sample from Nyandarua, lane 2- a sample from Nakuru, lane 3- a sample from Meru, lane NC- Negative control)

Molecular identification of PCN species

The polymerase chain reaction (PCR) amplicons were observed at 434 bp in all the cyst isolates from Nyandarua, Nakuru and Meru. This indicates that the species detected was *G. rostochiensis* in all the samples except the ones that did not amplify (Figure 3.3). Primer (ITS5/PITSr3) specific for *G. rostochiensis* amplified 23 (82.1 %) of the samples from Nakuru county, 26 (86.6 %) of the samples from Meru county and 27(90 %) of the samples from Nyandarua county. No PCR reactions were detected in 14 (15.5 %) samples.

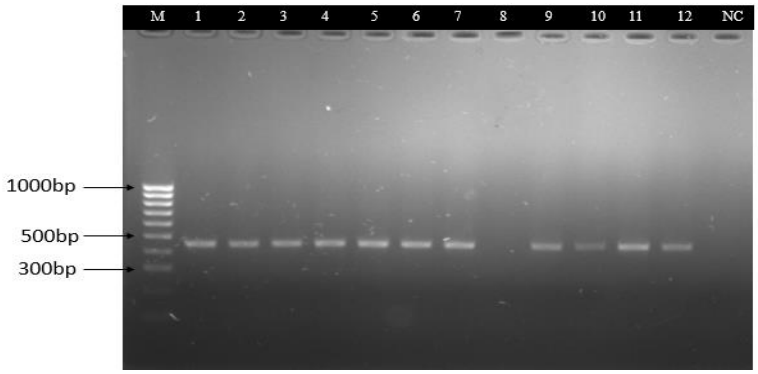

Figure 3.3. Multiplex PCR using primers ITS5, PITSr3 and PITSp4

Lane M-100 bp Molecular marker, Lane 1-3 samples from Meru county (UH3, UH2, LH), Lane 4-6 samples from Nakuru county (samples UH3, UH2, LH4), Lane 7-12 samples from Nyandarua county (2 samples in UH3, 2 samples in UH2, 2 samples in LH4), Lane 8 Absent, Lane NC-negative control

3.5.2 Genetic diversity of PCN populations using microsatellite markers

3.5.2.1 Microsatellite development and marker polymorphism

Thirteen out of the 20 loci produced amplicons. Among the 13 loci, 9 appeared to be monomorphic. Only 4 loci Gr67, Gr79, Gr90 and GRM2 were polymorphic (Table 3.1). Among the four polymorphic markers, locus GRM2 was designed for this study. Seven out of the 20 loci (35%) failed to produce amplifications and were therefore excluded from the study.

Genetic analysis of SSR markers and *G. rostochiensis* populations

The four alleles displayed allelic variations. A total of 2 alleles were observed per locus. The highest allele frequency rate of 0.8 and the lowest allele frequency rate of 0.2 for locus Gr67 were registered in samples collected from Nyandarua (Figure 3.4).

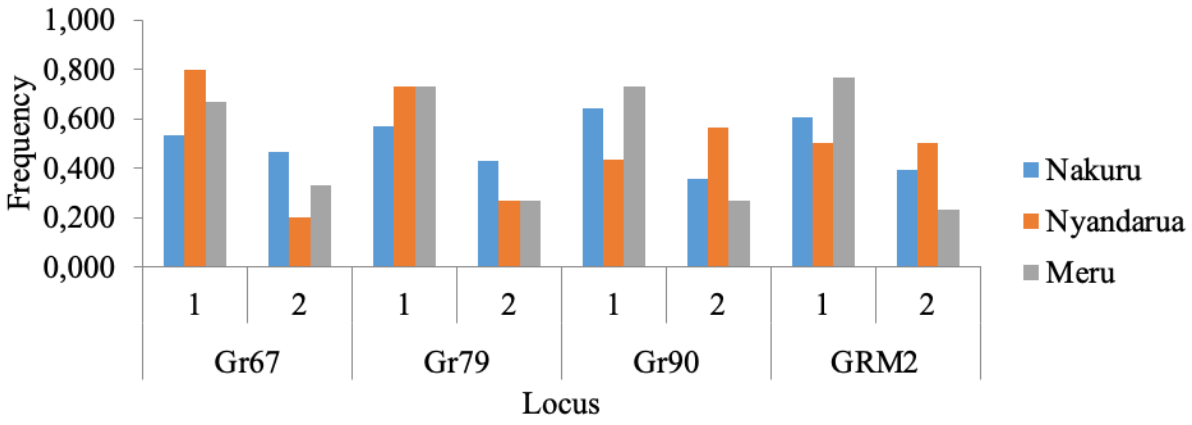

Figure 3.4. Allele frequency by locus for the three populations obtained from Nakuru, Nyandarua and Meru counties

The results in figure 3.4 and table 3.5 showed that there was genetic variation among the 88 samples analyzed. The observed allelic richness (*A*r) across the three counties ranged from 1.471 to 2.00. The expected heterozygosity (*H*e) and Shannon's index (*I*) across the three samples varied from 0.325 to 0.500 and 0.500 to 0.693 respectively. The mean expected heterozygosity for all the populations ranged from 0.436 to 0.482 (Table 3.5). There was no significant difference on the *H*e and *I*, thus indicating a narrow range of genetic diversity. All the loci exhibited significantly high F_{IS} values of 0.109 for Gr67, 0.131 for Gr79, 0.217 for Gr90 and 0.144 for GRM2 (Table 3.5). The PIC values of the locus for all the PCN populations were 0.344 for Gr67, 0.340 for Gr79, 0.364 for Gr90 and 0.359 for GRM2 (Table 3.5). According to the classification of PIC by Botstein *et al*. (1980) all the four selected loci demonstrated an intermediate polymorphism (PIC ≥ 25) on samples collected from the three counties (Table 3.5).

The PCN sample populations from Nakuru had the highest heterozygosity of 0.481, whereas Meru PCN samples had the least heterozygosity of 0.396. The mean number of alleles in the PCN populations from the three counties Nakuru, Nyandarua and Meru were 2.00 (Figure 3.5.). The private and locally common alleles were not detected in the three populations (Figure 3.5.).

Table 3.5. Genetic diversity indices for each loci and PCN populations collected from Nakuru, Nyandarua and Meru counties

Population	Population indices	Loci			
		Gr67	Gr79	Gr90	GRM2
Nakuru	NS	28	28	28	28
	Na	2	2	2	2
	*A*r	1.990	1.960	1.849	1.912
	I	0.691	0.683	0.652	0.670
	He	0.497	0.490	0.459	0.477
Nyandarua	NS	30	30	30	30
	Na	2	2	2	2
	*A*r	1.471	1.642	1.965	2.000
	I	0.500	0.580	0.684	0.693
	*H*e	0.320	0.391	0.491	0.500
	uHe	0.325	0.398	0.499	0.508
Meru	NS	30	30	30	30
	Na	2	2	2	2
	*A*r	1.800	1.642	1.642	1.557
	I	0.637	0.580	0.580	0.543
	He	0.444	0.391	0.391	0.358
For all populations	NS	88	88	88	88
	He	0.444	0.436	0.482	0.471
	PPl (%)	100	100	100	100
	*F*IS	0.109	0.131	0.217	0.144
	PIC	0.344	0.340	0.364	0.359

NS: number of samples; Na: alleles number, *A*r: allelic richness; *H*e: expected heterozygosity; *I*: Shannon information index; PPL: the percentage of polymorphic loci, PIC: polymorphism information content *F*IS: deviation from random mating.

The AMOVA results showed that only two levels (among and within populations) contributed to the overall genetic variation. The results revealed that 96% of genetic variance came from within population and 4% of genetic variation of PCN population came from among the population. Therefore, the genetic variance within the population was higher than between the population (Table 3.6). The two components of AMOVA were highly significant ($P < 0.001$) (Table 3.6).

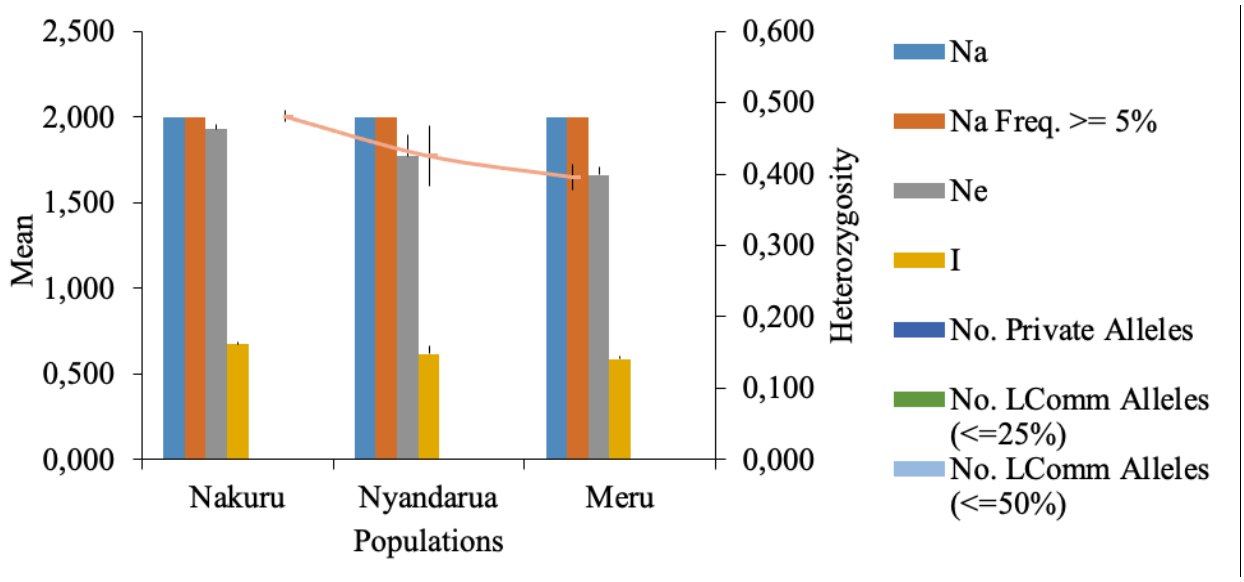

Figure 3.5. Allelic patterns across populations
Na: number of observed alleles; Na Freq: Allele's frequency; Ne: effective number of alleles, I: Shannon information index, No. private alleles: Number of private alleles, No. LComm Alleles: Number of local common alleles

Table 3.6. Analysis of molecular variance for potato cyst nematode (PCN) samples from Nyandarua, Nakuru and Meru, Kenya

Source	df	SS	MS	Est. Variance	% of total variation	P value
Among populations	2	8.020	4.010	0.038	4	0.001
Within populations	85	152.457	1.794	0.897	96	0.001
Total	175	160.477	5.804	0.935	100	

Note: df: degrees of freedom; SS: sum of squares; MS: means of a square; Est. Var: variance component estimate; %: percentage of total variation

Pairwise genetic differentiations

The fixation index (*F*st) ranged from 0.021 to 0.048, indicating little genetic differentiation (*F*st < 0.05) among the three *G. rostochiensis* populations (Table 3.7).

Table 3.7. Genetic differentiation (*F*st) among *Globodera rostochiensis* specimens obtained from Nakuru, Nyandarua and Meru counties, Kenya

	Nakuru	Nyandarua	Meru
Nakuru	0.000		
Nyandarua	0.041	0.000	
Meru	0.021	0.048	0.000

*F*st < 0.05 = little genetic difference; *F*st = 0.05–0.15 = moderate genetic difference; *F*st = 0.15–0.25 = great genetic difference; *F*st > 0.25 = very great genetic difference, according to Hartland Clark (1997)

Genetic relationship of *G. rostochiensis* populations

The 88 PCN samples formed two major clusters 1 and 2 (Figure 3.6). Cluster 1 had 23 PCN samples having two sub-structures (1.1 and 1.2). While cluster 2 had a high number of PCN samples (65) and had 3 sub-groups (2.1, 2.2 and 2.3). Sample 77 from Meru had its own sub-structure 2. This could be of unique genetic evolution (Figure 3.6.). Each cluster was composed of different populations emanating from the three counties (Nyandarua, Nakuru and Meru).

Genetic structure of PCN samples from Nyandarua, Nakuru and Meru

The principal coordinate analysis PCoA analysis classified the PCN populations into three groups (Figure 3.7). The samples did not cluster according to counties for example, all the three clusters had samples from all the three counties (Nyandarua, Nakuru and Meru). According to PCoA, out of the 88 PCN samples, 16 unique genotypes were identified. The percent variance accounted for by population I, II, III was 27.73 %, 28.89 %, and 24.42 % respectively (Figure 3.7 and Table 3.8). The total percentage of variation of all the 88 samples was explained by the first three axes which was 81.05 %. The Eigen values of the four axis were 9.943, 9.543, 8.404 and 6.520, respectively. All the axes had Eigen value >1 making them highly informative.

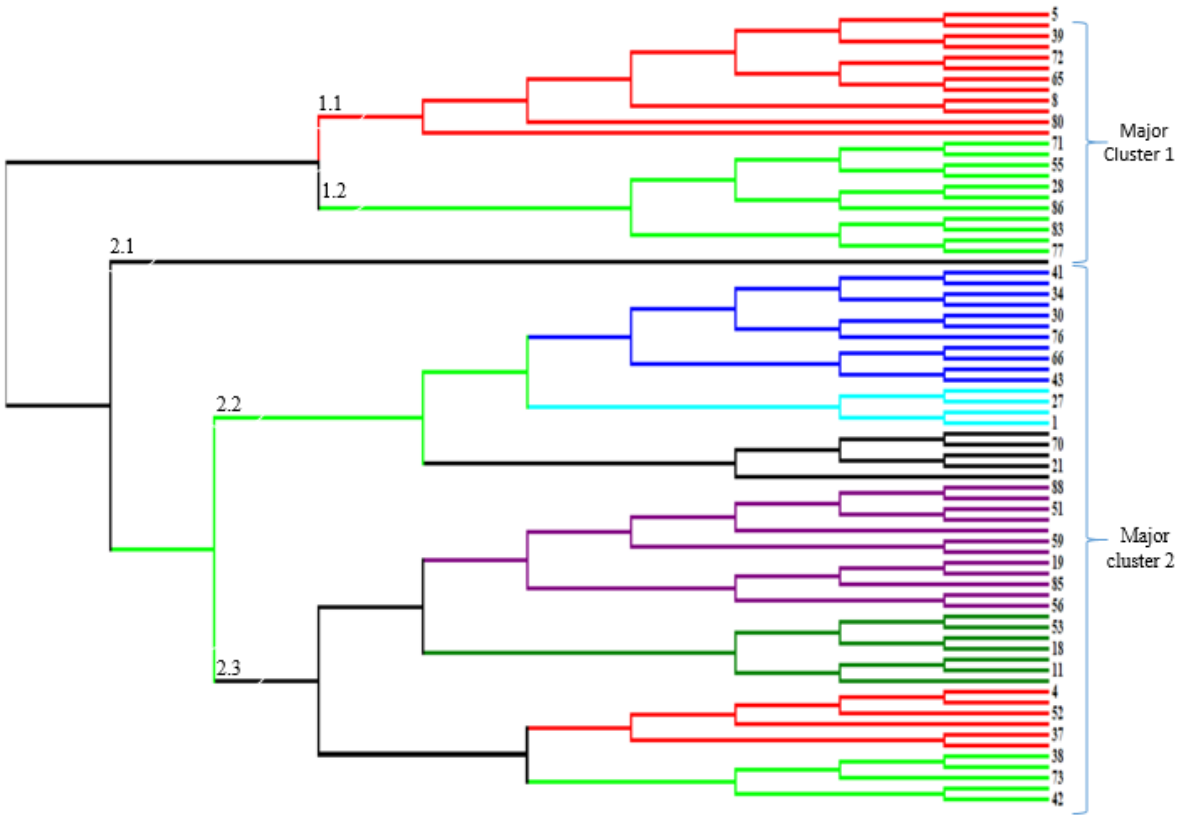

Figure 3.6 UPGMA dendogram of 88 PCN samples belonging to *G. rostochiensis*

(Sample 1-28 were samples from Nakuru county, Samples 29- 58 were samples from Nyandarua county, Samples 59-88 were samples from Meru county)

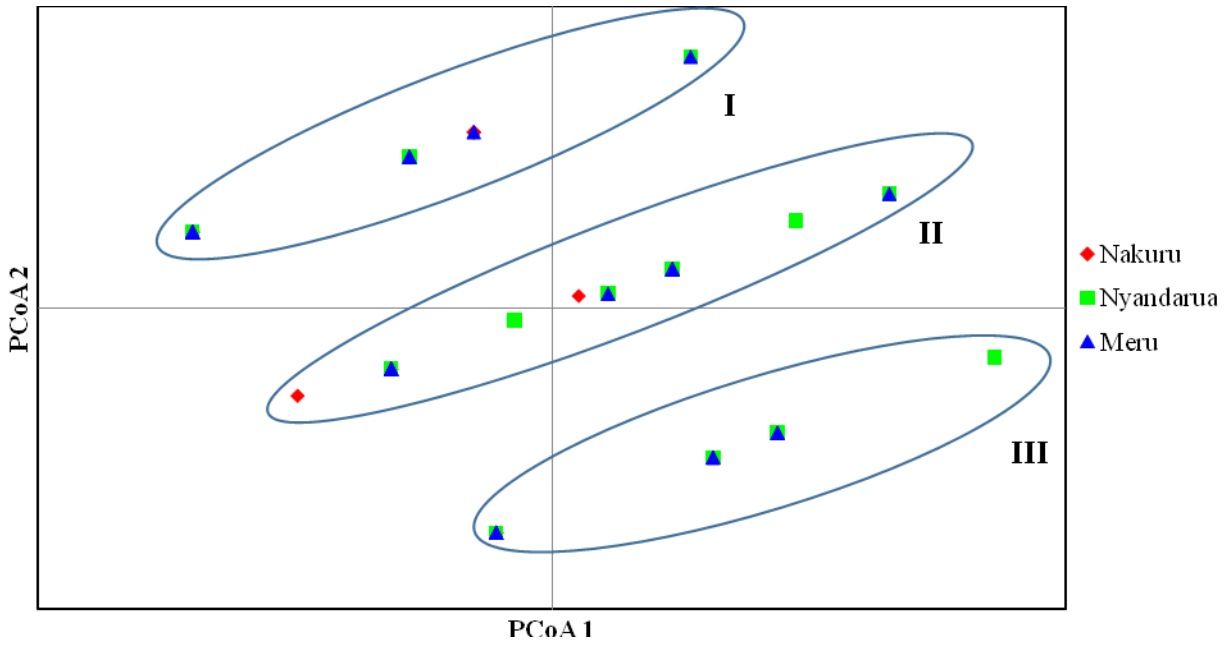

Figure 3.7. PCoA results of 88 PCN samples using four microsatellite markers

Table 3.8. Eigen values and total variation of six principal components for 88 PCN samples

Axis	Eigen value	% of variance	Cummulative % of variance
1	9.943	28.89	28.89
2	9.543	27.73	56.63
3	8.404	24.2	81.05
4	6.520		

Based on the highest K value obtained by the structure harvester (K=8.25), the population structure analysis revealed six distinct genetic structures (K=6) (Figure 3.8 and 3.9). The bar plot model of clustering revealed that all the populations evaluated were admixtures. Each of the individual populations was denoted by 6 distinct group of colours red, green, blue, yellow, pink and blue.

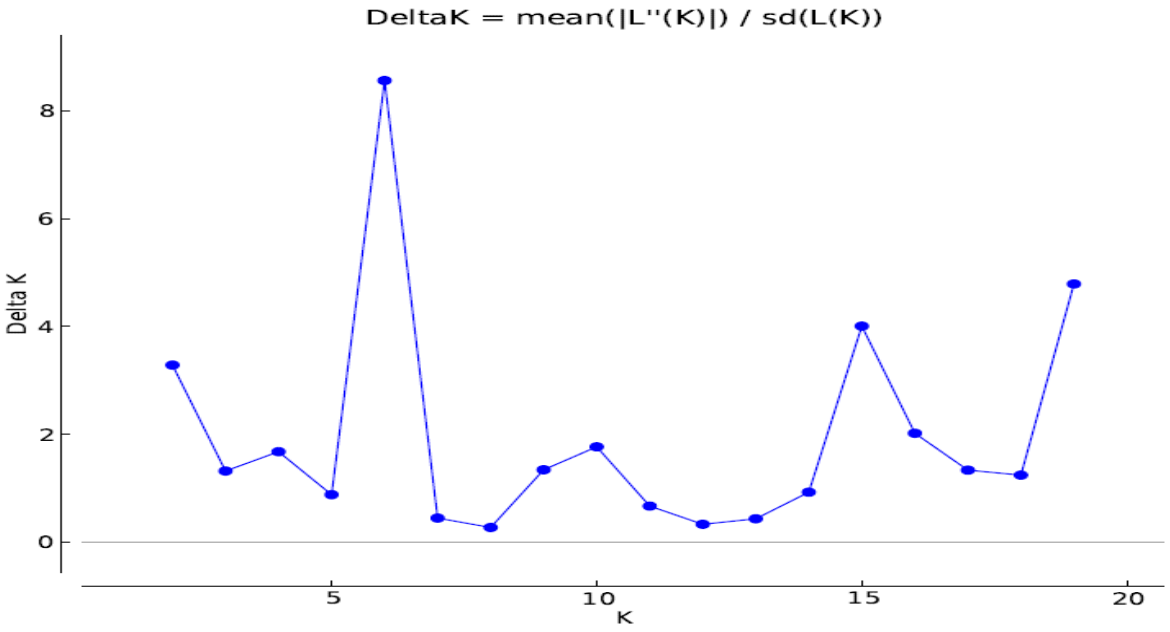

Figure 3.8. Plot of Δ*K* used to determine the most likely number of genetic structures (*K*) of PCN populations

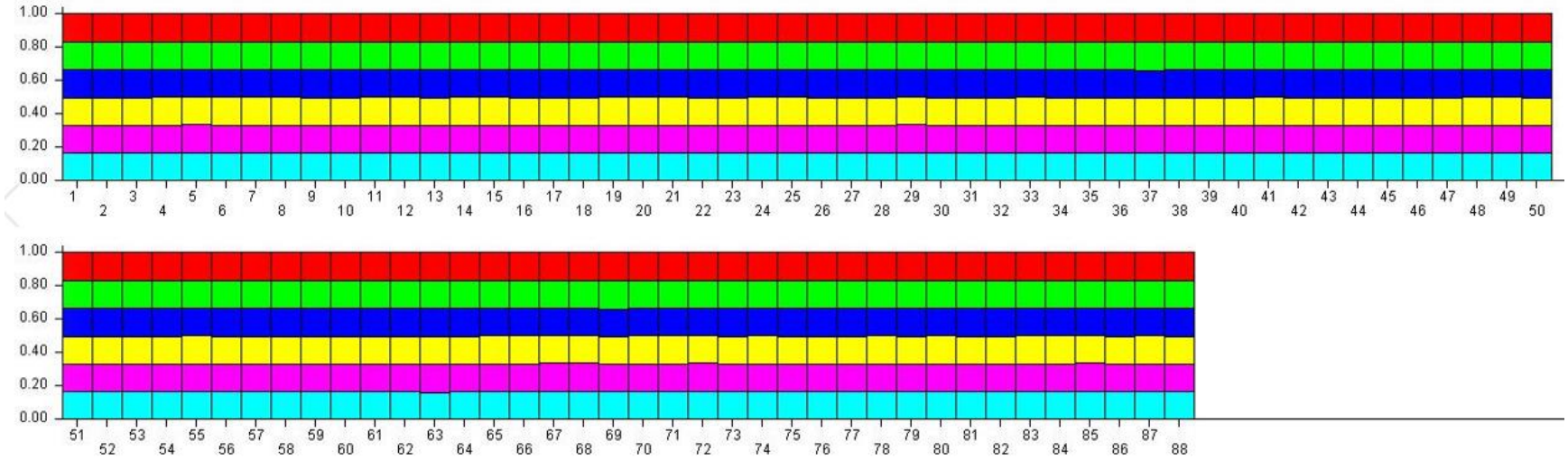

Figure 3.9. Bayesian clustering analysis (STRUCTURE) performed on 88 PCN samples

Each vertical column represents an individual with a genome divided into six genetic clusters (represented by the six different colors). Individuals from the various populations shown above are separated by vertical bars. All the 88 PCN were assigned to 6 genetic clusters

3.6 Discussion

This study revealed that morphometric measurements were overlapping to an extent where they cannot be relied upon to identify PCN species conclusively. Therefore, for a more successful and accurate identification of PCNs it was imperative to apply molecular analysis to clear doubts and confirm the results. Using molecular analysis *G. rostochiensis* was present in all of the soil samples, while *G. pallida* was absent. The absence of *G. pallida* in the three counties namely Nakuru, Nyandarua and Meru could be as a result of their preference to low temperature below 10 ^{o}C which favours reproduction unlike *G. rostochiensis* which reproduces well in temperatures ranging from 11.5 ^{o}C to 32.5 ^{o}C (Jones *et al.,* 2017; Kaczmarek *et al.*, 2012).

To explore intra population genetic diversity among the *G. rostochiensis* populations in Kenya microsatellite markers were used. The SSRs markers selected for this study demonstrated that they were polymorphic (PIC $\geq$ 25) among the PCN populations from the three Counties. The F_{IS} value revealed that the three *G. rostochensis* populations from Nyandarua Nakuru, and Meru were inbreeding. Additionally, the genetic differentiation pairwise matrix showed insignificant differentiation (Fst < 0.05) in all the populations. This could be as a result of relative close proximity of the study areas in addition to the polyandry mode of reproduction and human activities (Picard *et al.,* 2004). According to Mwangi (2019), farming practices such as recycling of seeds, use of farm implements and footwear without cleaning and transportation of seed with soil could accelerate spread of PCN resulting in gene flow *G. rostochiensis* across the potato growing counties in Kenya.

The result of this investigation showed that every populations exhibited significantly high heterozygote deficiency therefore deviating from Hardy-Weinberg equilibrium. It is hypothesized that the deficiency in the populations is as a result assortative mating and limited dispersal of J2s to few centimeters or decimeters. According to Wang *et al*. (2017), only J2s of *Heterodera* spp. and *Globodera* spp. are mobile so dispersal is very low. As a result, given the low dispersal of *G. rostochiensis* there is a high likelihood of individuals mating with one another, and therefore the population becomes either siblings or half siblings (Picard *et al.,* 2004). Furthermore, the assortative mode of reproduction is common in organisms characterized with limited dispersal and would result in reduction in genetic variability (Picard *et al.* 2004). Previous studies have found heterozygote deficiency in cysts forming nematodes *Heterodera schachtii* (Plantard and Porte

2004), *G. tabacum* (Alenda *et al.*, 2014), *Globodera pallida* (Picard *et al.*, 2004), *H. avenae* (Wang *et al.* 2015).

The analysis of molecular variance (AMOVA) in this study showed that variation within the population accounted for the majority of the diversity across all the counties. These findings suggest that the Kenyan PCN population could be from one origin. This could be as a result of spread of PCN across the counties through uncertified seeds. The PCoA analysis of genetic diversity structure showed that, PCN populations were in three genetic clusters. Moreover, the samples from the three counties clustered and intermixed within the three populations in the PCoA analysis. The mixing of populations from different counties could be as a result of gradual migration of PCN populations, primarily as a agricultural practices that may have contributed to passive dispersal such as transfer of planting material among the counties (Alenda *et al.*, 2014).

The phylogenetic tree in this study confirmed the intermixing of populations from the three different regions of collection based on Nei's genetic dissimilarity. Despite their different origins, the three populations have a close genetic relationship, suggesting that they may have evolved together. In the phylogeny tree, samples that were placed closer together suggest that they were genetically more similar, whereas samples that were placed further apart indicated that they were genetically dissimilar despite being from the same region. Natural mutation and natural selection within the population may have contributed to this genetic variance. This was also reported by Mwangi (2019) who observed that Kenyan PCN populations formed single cluster when compared with samples from Germany.

The current genetic structuring of the present study revealed there was gene flow between the populations, this was shown by the intermixing of the PCN populations. The relationships between population structures with geographical origin were indistinct. This could be as a result of close proximity of the regions. Furthermore, cyst nematodes practice polyandry fertilization, genetic diversity within a single cyst having hundreds of individual's eggs may occur (Mimee *et al*., 2015). In Western Europe, genetic variability of *G. pallida* was reported to occur in a single plant, a field and an entire region (Plantard *et al.*, 2008).

Overall, this study found that gene flow occurred not only within counties but also between counties. According to McDonald and Linde (2002), potato cyst nematode should be classified as

a pest with a high capacity of overcoming the resistance because of the polyandry reproduction and high mutations which allows gene variability and those species with mutant genes may have selection/survival advantage. It is therefore important to avoid introduction of PCN from other clades since they would very divergent requiring additional resources to manage and would enhance high adaptive potential of the current populations. To limit spread, measures such as using certified seeds and cleaning farm implements and footwear should be promoted.

CHAPTER 4: FACTORS CONTRIBUTING TO SPREAD AND POPULATION BUILD-UP OF POTATO CYST NEMATODES (*Globodera spp.)* IN SMALLHOLDER FARMS IN NYANDARUA COUNTY-KENYA

4.1 Abstract

Potato cyst nematodes (PCN) pose a serious threat to potato production in Kenya. A study was conducted to identify factors that may be contributing to the spread and increase in population density of the nematodes in smallholder farms in three agro-ecological zones; Upper Highland-3 (UH3), Upper Highland-2 (UH) and Lower Highland (LH4) of Nyandarua county in central Kenya. The study's sample of 65 farms was drawn using a stratified random sampling procedure. A total of 62 (95.1%) samples out of 65 were infested with PCN. A significantly ($p \leq 0.05$) lower PCN count of 63.1 cysts were recorded in Lower Highland (LH4) compared to 203.7 and 289.2 cyst/300 g of soil in the higher zones (UH2 and UH3), respectively. Sandy clay loam soil had a significantly ($p \leq 0.05$) higher number of cysts compared to sandy clay soils. Farms where crop rotation was practiced had lower PCN infestation than those that relayed cropped potatoes. Nematode numbers were higher, up to 302 cysts/300 g, in farms growing more than one potato crop cycle in a year, compared to 197 cysts/300 g in farms where one potato crop cycle was practiced per year. Only 15% of the farmers used certified seed while the rest used farm-saved tubers or seed bought from the local markets. A commonly grown variety-Shangi had significantly ($p \leq 0.05$) high cyst counts that ranged from 174.3 to 463.1 cysts/ 300 g. The number of cysts recovered from soil adhering to farm tools, footwear and potato tubers ranged from 2.6-13.8 cysts /50 g soil, 1.8-8.8 cysts /50 g soil and 2.8-12.9 cysts /50 g soil respectively. Due to the limited knowledge of available strategies, none of the farmers demonstrated any elements of integrated pest management (IPM) strategies. These findings re-affirm the need for an integrated strategy that include crop rotation, planting of disease-free tubers, resistant varieties, field sanitation as well as enhancing farmers' knowledge on management of the pest to reduce the spread and build-up of PCN.

4.2 Introduction

Potato cyst nematodes (PCN), *Globodera* spp., are a potential threat anywhere potatoes are grown. They are specialized pathogens of solanaceous species and economically important plant parasitic nematodes of potato (Sabeh *et al.,* 2019). It is estimated that two species, *G. rostochiensis* and *G. pallida*, account for 9 % of global potato yield loss (Turner and Subbotin, 2013). However, if no control or containment strategies are implemented, total loss in potato production can occur. Skantar *et al.* (2011) discovered a potential third species, which was later described as *G. ellingtonae* by Handoo *et al*. (2012). Its pathogenicity to potato on the other hand varies (Zasada *et al.,* 2019).

The pests were first reported in the Andes of South America, from where they spread to Europe, Asia, North Africa, and Western America (Mai, 1977). Both *G. rostochiensis* and *G. pallida* were most likely introduced and established in Europe in the 1850s as a result of the Irish potato famine, when tubers contaminated with PCN were collected and brought to Europe as breeding material for late blight (*Phytophthora infestans*) resistant potato varieties (Evans *et al.,* 1975). Damage to potato crops by nematodes was observed in Germany in the 1880s and in the United Kingdom in the early 1900s (Evans *et al*., 1975). Since then, PCN has spread to nearly every country where potatoes are grown, primarily via contaminated seed potatoes. To date, PCN has been found in 126 countries so far 79 countries with *G. rostochiensis* and 55 with *G. pallida*) (CABI, 2020). Potato cyst nematode is a new pest in East Africa, with *G. rostochiensis* being discovered in Kenya in 2014 (Mwangi *et al.,* 2015) and Uganda and Rwanda in 2018 (Cortada *et al.*, 2020, Niragire *et al.*, 2019). Previously surveys in Kenya revealed that the pest has a wide distribution in the potato growing counties (Haukeland, 2016; Mburu *et al.*, 2020).

There are numerous site-specific factors that contribute to the spread and population build-up of PCN in smaller holder farms worldwide. These site-specific factors include soil characteristics, cultural practices, human activities and weather (Avendano *et al.,* 2004). Depending on the site specific factors PCN can take 1 to 3 months to complete life cycle (Mwangi *et al.*, 2021; Abd and Askary, 2015; Lilley *et al.,* 2005). In addition to the short life cycle, the rapid multiplication is an important aspect in helping the pest to maintain a high population threshold, complicating its

management. Dixit (2019) noted that soil characteristics, cultural practices, host crop, and temperature all play significant in passive dispersal and build-up of plant parasitic nematodes.

Distribution and densities of PCN are heavily influenced by soil characteristics (Compton, 2013). According to Fajardo *et al.* (2011), sandy soils in vineyard had significantly higher population density of plant parasitic nematodes because of their ease of mobility and infectivity when compared to clay vineyard soils. According to Mburu *et al.* (2020), the regular use of own seed may encourage and perpetuate the spread of PCN. Phillips (1989) reported that rotational intervals of five years or more are required in the absence of other management methods to prevent unnecessary yield losses due to PCN infestations.

According to Gartner *et al.* (2021), tolerant cultivars planted in infested soil can increase PCN population levels while still yielding acceptable yields, thus thwarting PCN management programs. An increase in PCN population was observed by Dandurand *et al.* (2019) when a susceptible host was planted, noting that if PCN is not controlled, dramatic increases in population can occur. Good farm hygiene/sanitation that includes cleaning of farm tools and equipment will aid in reducing spread from one field to the other (Price *et al.,* 2021). Trap cropping has been shown to lower cyst nematode populations by up to 80% (Chitambo, 2019; Scholte, 2000). Dewar *et al.* (2000) demonstrated that PCN population might increase by up to 148% in one year with one volunteer plant per m^2. Soil fumigants have been used successfully to lower PPN population below the economic threshold level (Zasada *et al.*, 2010). However, several of them including methyl bromide, aldicarb, and fenamiphos have been phased out of regular usage because of their harzardous effect on the environment (Desaeger *et al.*, 2021; Sasanelli *et al.,* 2021).

Although it has been established that PCN is widespread in Kenya's major potato growing counties, information on factors contributing to the pest's spread and build-up of population density is scanty. This study's objective was to examine the relationship between PCN spread, population density of PCN and site-specific factors, with a focus on climatic conditions, soil characteristics and cropping practices, that could have influenced the prevalence and population densities of PCNs Nyandarua county.

4.3 Material and methods

4.3.1 Study area

The study was conducted in Nyandarua county, Kenya located at longitude 00^0 55` East and latitude 036º 62` South. The county was chosen for the study for a variety of reasons, including: (i) its importance in potato production, (ii) its long history of growing potatoes (iii) its diverse cropping systems (iv) reported incidence of PCN and (v) the variety of agro-ecological zones in which potatoes are grown. The three main agro ecological zones (AEZs) include: upper highland (UH3) represented by Kinangop sub-county, upper highland (UH2) represented by Ol jororok sub-county and lower highland (LH4) represented by Kipipiri sub-county formed the agro-ecological focus of the study. The study sampling sites received an average annual rainfall between 1000-1500 mm in UH3, 1000-1250 mm in UH2 and 800-1000 mm in LH4. The annual temperatures ranged from 11-20 ºC in UH3 and UH2 and 15-25 ºC in LH4. The altitude of the three AEZs ranged from 2500-2700- meters above sea level (masl) in UH3 while in UH2 2370-2430 masl and in LH4 1299 to 2280 masl.

4.3.2 Sampling procedures

Farmers were chosen at random using stratified sampling techniques from a list of potato producers obtained from the local agricultural extension offices from three sub counties namely Oljororok, Kinangop and Kipipiri. The sample size was calculated using the following formula by Cochran (1977):

$$n = \frac{Z^2PQ}{E^2}X100$$

Where n is the sample size, Z is 95% confidence level (standard deviation of 1.96), P is the prevalence of PCN reported in the area which is 90% (Haukeland, 2016), Q is P-1 and E is margin of error 8%.

4.3.3 Field survey

A questionnaire that was used to capture information on site specific factors that enhance spread and population build-up was developed. Questions on farmer's knowledge on PCN, potato varieties grown, source of planting material, prevalence of diseases, cropping systems, and potato cycles in a year were developed. The questionnaire was pre-tested with ten farmers in UH3 before

being revised accordingly. The final version included both open-ended and closed-ended questions. Farmers' responses were cross-checked with field observations during interviews conducted in their potato fields. A total of 65 farmers were interviewed, with 25 from UH3, 20 from UH2 and 20 from LH4. During farm interviews, occurrences of bacterial wilt (*Ralstonia solanacearum*), viruses (potato leaf roll virus (PLRV), and potato mosaic, and late blight (*Phytophthora infestans*) were recorded as present or absent.

4.3.4 Soil sample collection

Soil samples were collected from 65 farms where the field survey was conducted in the three AEZs (25-UH3, 20-UH2 and 20-LH4) using a soil auger. The samples were taken from the potato rhizosphere at a depth of 0-30 cm of ten plants at maturity stage selected at random. The sub samples from each farm were combined to form a composite sample of about 600 g. To test factors contributing to spread a total of 10 soil samples per farm were collected from hoe (an agricultural tool commonly used for digging and weeding), seed potatoes and footwear (gumboots and shoes) from each farm store. Global positioning system (GPS) readings were collected at the sampling locations and used as a geo-reference point for the sample collection and the interview sites. Using ArcGIS 10.2.2 computer software, geographical coordinates for each field were mapped.

4.3.5 Cysts extraction and quantification of PCN levels

Soil samples collected were tested for PCN by thoroughly mixing the composite sample from each farm, after which 300 g was collected and determining the presence of cysts using the Fenwick can method (Fenwick, 1940). The extracted cysts were then picked and counted under a dissecting microscope (Leica microscope M 275 with KL 1500 illuminator) at 10X. Three cysts were crushed and eggs were counted on a counting dish under a dissecting microscope (Leica microscope M 275 with KL 1500 illuminator) at 10X and 40X magnification. Cysts were expressed as numbers per 300 g while the egg counts were recorded per cyst.

4.3.6 Soil chemical and physical analyses

Two hundred grams of the collected soil samples from each of the 65 farms were analysed for physical and chemical characteristics. This was done at the physical and chemistry laboratory

of the University of Nairobi (UoN), Department of Land Resource Management and Agricultural Technology (LARMAT).

Chemical analysis: Soil samples were subjected to chemical characterisation using the following protocols: Soil organic carbon (SOC) was determined using Walkley –Black method (Nelson and Sommers, 2018); total nitrogen (N) was determined using micro Kjeldahl's digestion-distillation method (Black, 1965; Kjeldahl, 1883) and available phosphorous by the molybdenum blue colorimetric method using a $HClO_4$-H_2SO_4 solution for digestions (Qaswar *et al.,* 2019). Absorption spectrometry was used to determine the availability of soil elements such as manganese (Mn), potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) (Sims, 1991). The calorimetric method was used to determine trace elements in soil (Fe, Zn, and Cu). The pH of the soil was determined using a Hanna pH meter with a glass electrode (Blanchar, 1988). Electrical conductivity (EC) was measured as dSm^{-1} as described by Oztan and Ulgen (1961).

Physical analysis: A subset of the 65 soil samples was used for physical analysis to assess relationship between soil texture and PCN population densities. Soil particle size analysis was conducted for each of the samples from the three agro-ecological zones using the modified hydrometer method (Gee, 1986). The Bouyoucos method was used to determine the proportions of clay (< 2 µm), silt (2.1–49 µm) and sand (>50 µm) (Phogat *et al.*, 2015).

4.4 Data analysis

The infestation levels of cyst and eggs were classified according to Jones (1970) and Mburu (2020) as follows: absent (0 cysts/100g soil and 0 eggs/g soil), low (1-9 cysts/100g soil, 1-14 eggs/g soil), moderate (10-19 cysts/100 g soil, 15-30 eggs/ g soil), fair (20-39 cysts/100 g soil, 31-60 egg/ g soil), fairly high (40-80 cysts/100 g soil, 61-120 eggs/ g soil), severe (over 80 cysts/100 g soil, over 121eggs/g soil). The infestation levels of eggs and cysts were mapped using GIS mapping software (Arc-GIS). Dots having a 5-color scheme matching to cyst and egg density were used to project to corresponding sampling sites.

Statistical Package for Social Sciences (IBM SSPS) version 26 computer program was used to analyze survey data using descriptive statistics, data explorations, and cross-tabulations. In relation

to soil characteristics and farming practices, parameters on nematode frequency of occurrence, mean population density in 300 g soil, relative density, and greatest density (maximum value from the range of population density in 300 g soil) were calculated. Potato cyst nematode prevalence was assessed using the formula;

$$\text{Prevalence of potato cyst nematodes (\%)} = \frac{\text{Number of farms having PCN}}{\text{Total Number of farms}} \text{X } 100$$

Correlation analysis was used to determine the relationship between PCN population density and soil characteristics.

4.5 Results

4.5.1 Farmers' knowledge of potato cyst nematode

A total of 40 (61.5%) farmers out of 65 were not aware of PCN as a potential threat to potato production. Out of the 65 farmers interviewed, 25 had some knowledge on the pest (Figure 4.1). While none of the farmers had knowledge of integrated pest management (IPM) strategies for the pest.

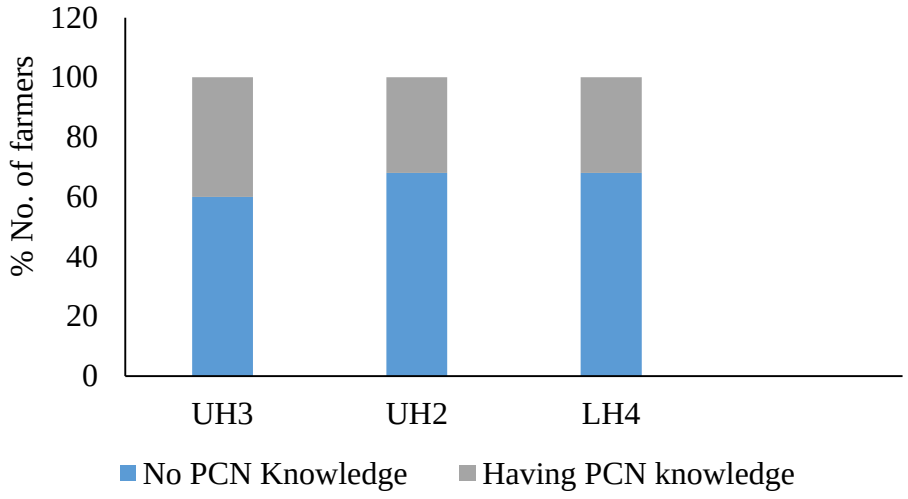

Figure 4.1. Farmers' knowledge of potato cyst nematodes in three agro-ecological zones in Nyandarua county

4.5.2 Distribution and population density of PCN in different AEZs

The PCN infestation levels of the sampled farms varied from 3.5 % absent (0 cyst), 40.0 % low (1-9 cysts), 6.2 % moderate (10-19 cysts), 4.2 % fairly high (20-39 cysts), 21.5 % high (40-80 cysts), and severe 24.6 % (≥80) in 100 g^{-1} of soil. Detection of viable PCN eggs varied from absent (0 eggs) at 12.3 % farms, moderate (1-30 eggs) 4.2 % farms, fair (31-60 eggs) 6.2 %, fairly high (61-120 eggs) 18.5 %, high (121-240 eggs) 18.5 %, severe ≥240 40 % eggs in $g^{-1}$of soil (Figure 4.2). In all the three AEZs, the population density with more than 240 eggs g^{-1} soil (40 % of infested sites) and that with 1-9 cysts /100g soil (40 % of infested sites) was the most prevalent.

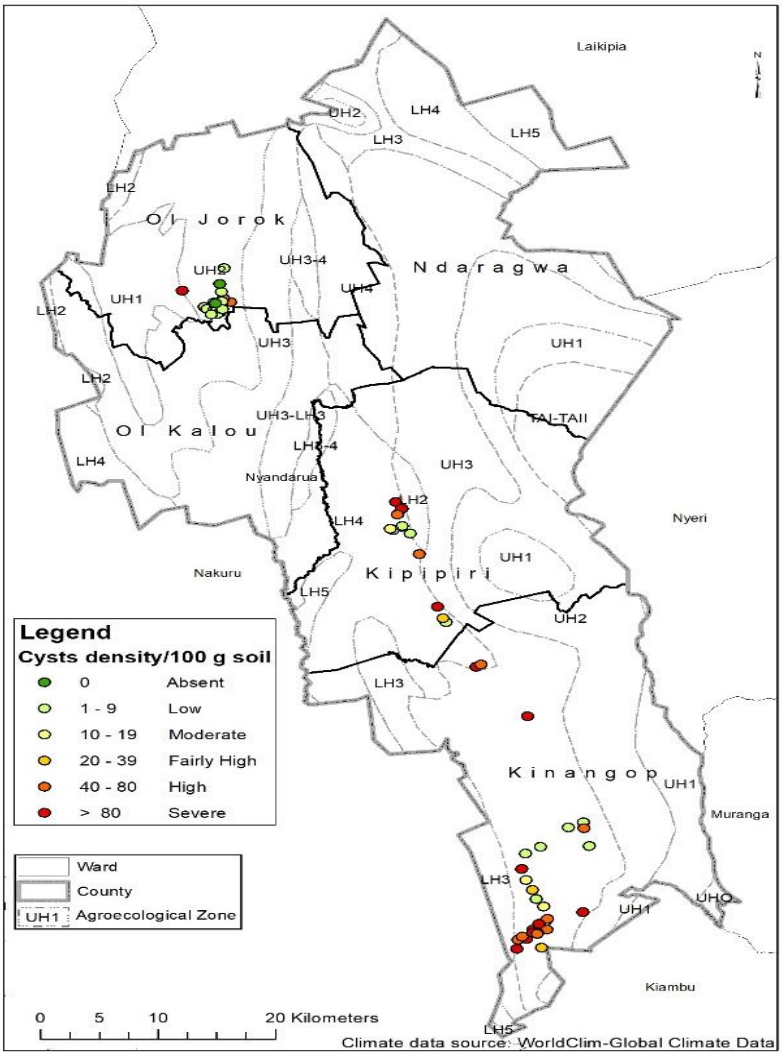

Figure 4.2. Geographical distribution level of PCN cyst infestation in Nyandarua county

4.5.3 Occurrence and population density of PCN infecting potato farms

Potato cyst nematodes were recovered from 62 farms (95.4 %) of the 65 samples collected. A high PCN prevalence of 100 % was observed in UH3 and LH4. In all the three AEZs, the cysts count ranged from 0-1037 cysts /300 g of soil. The population density of PCN was not significantly different in UH3 and UH2 with each having 289.2 and 203.7 cysts /300 g of soil, respectively but were significantly high compared to LH4 which had 63.1 cysts /300 g soil (Table 4.1).

Table 4.1. Potato farms infested by potato cyst nematodes (%) and their population density in three agro ecological zones (AEZ) in Nyandarua county, Kenya

AEZ	Samples analyzed	Number of samples infested	Percentage potato farms infested by PCN	Average number of cyst/300 g soil
Upper Highland 3	25	25	100	289.2b
Upper Highland 2	20	17	85	203.7b
Lower Highland 4	20	20	100	63.1a
$LSD_{(0.05)}$				101
Total	65	62	95.4	186.7

Means within columns with the same letter are not statistically different from one another ($p \leq 0.05$)

4.5.4 Relationship between PCN densities with temperature, rainfall level and altitude

A significantly ($p \leq 0.05$) lower PCN count of 63.1 cysts/300 g was recorded in LH4 where rainfall was less than 1000 mm and the temperature range was 15-25^{o}C compared to 289.2 and 203.7 cysts/300 g in UH3 and UH2 respectively where the annual rainfall was greater than 1000 mm and temperature was between 11-20^{o}C (Table 4.2).

Table 4.2. Altitude, annual rainfall, ambient temperature in relation to PCN population density in three agro-ecological zones in Nyandarua county, Kenya

Sub-county	AEZs	Altitude (m.a.s.l)	Annual rainfall (mm)	Ambient temperature (^{o}C)	Mean cysts/300 g soil
Kinangop	UH3	2500-2700	1000-1500	11-20	289.2b
Oljororok	UH2	2370-2430	1000-1250	11-20	203.7b
Kipipiri	LH4	1299-2280	800-1000	15-25	63.1a
$LSD_{(0.05)}$					101

4.5.5 Effects of soil physical properties on potato cyst nematode population density

The soils in the study area were classified as clay loam, loam, sandy clay, sandy clay loam, sandy loam and clay. The sand fraction in the soils varied from 34 % (clay) to 54 % (sand clay loam). The proportion of sand was more in the sandy clay loam and sandy loam soil at 50-54%. A high proportion of silt of 32 and 33 % was registered in agro-ecological zones LH4 and UH2,

respectively. Clay soils had the highest clay particles (44 %) and the lowest sand particles (34%) in UH3 (Table 4.3).

The population density of PCN differed significantly with the different soil textural classes. Sandy clay loam soil had a significantly higher number of cysts/300 g of soil in all the three AEZs compared to other soil textural classes. The lowest mean cysts (2.0 cysts /300 g soil) were registered in sandy clay soils in UH2. A high PCN population density was recovered in soils having above 40% sand, >20 % silt and 28-37 % clay (Table 4.3).

Table 4.3. Soil particle size and classification of soil from three agro ecological zones (AEZ) and the corresponding potato cyst nematode population densities in Nyandarua county, Kenya

AEZ	Soil classification	Soil texture			PCN population density in 300 g soil				
		% Sand	%Silt	% Clay	Sample size	Mean	Standard Deviation	Minimum	Maximum
UH3	Clay	34	18	44	1	49.0		0	49.0
	Clay Loam	42	25	33	10	194.5	253.8	0.5	776.5
	Loam	44	24	32	2	214.5	39.6	186.5	242.5
	Sandy Clay	49	15	37	3	76.2	115.5	6.0	209.5
	Sandy Clay Loam	51	22	27	9	460.1	386.2	5.0	909.5
UH2	Clay Loam	39	29	32	4	96.9	105.1	4.0	288.0
	Loam	46	33	22	4	154.3	190.5	5.5	404.5
	Sandy Clay Loam	50	22	28	10	458.5	415.2	5.5	1133.0
LH4	Clay Loam	43	19	29	3	4.3	3.3	0.5	6.5
	Loam	45	32	23	10	107.2	202.4	2	615.5
	Sandy Clay	48	16	36	1	2.0		2.0	2.0
	Sandy Clay Loam	54	21	26	5	229.6	361.2	1.0	839.0
	Sandy Loam	51	28	18	1	177.0		177.0	177.0

Upper Highland (UH3); Upper highland (UH2); Lower Highland (LH4)

4.5.6 Effects of soil chemical properties on potato cyst nematode population density

PCN population had a significant ($p \leq 0.05$) positive correlation with organic carbon in UH3-r=0.265, nitrogen UH3-r=0.253, phosphorous LH4-r=0.205, calcium UH3-r=0.265, zinc LH4- r=0.223 manganese UH3-r=0.226 (Table 4.4). There was a negative correlation between PCN density with soil pH in UH3 - r=0.399, potassium UH3 - r=0.416, iron LH4 -r=0.340 calcium UH3 - r=0.464, copper UH3 - r=0.286 and magnesium UH3 -r=0.272 at $p \leq 0.05$ (Table 4.4).

Table 4.4. Correlation coefficients between potato cyst nematode (PCN) population density and soil characteristics, and soil nutrient indices in three agro ecological zones in Nyandarua county, Kenya

Soil parameters	UH3		UH2		LH4	
	PCN population density (r)	Soil nutrient indices	PCN population density (r)	Soil nutrient indices	PCN population density (r)	Soil nutrient indices
pH	-0.399*	5.02	0.060	5.49	0.149*	5.2
Electric Conductivity	-0.192*	0.393	-0.129*	0.282	0.116*	0.3
Organic Carbon	0.265*	3.22	0.207*	3.2	-0.069	3.8
Nitrogen	0.253*	0.315	0.048	0.306	0.001	0.369
Phosphorous	0.148*	93.95	-0.196*	31.79	0.205*	69.1
Potassium	-0.416*	1.97	0.082	2.84	0.079	2.57
Sodium	-0.077	0.416	0.192*	0.39	-0.040	0.399
Calcium	-0.464*	5.077	0.108*	6.38	0.027	6.52
Magnesium	-0.272	2.486	0.019*	2.64	0.023	3.47
Exchangeable Acidity	0.220*	1.788	-0.015	1.66	-0.085	1.85
Zinc	-0.205*	24.64	0.034	21.42	0.223*	18.97
Copper	-0.286*	1.74	-0.049	2.22	0.112*	2.05
Iron	0.148*	130.47	-0.259*	75.22	-0.340*	94.11
Manganese	0.226*	153.71	-0.240*	105.9	-0.277*	111.6

*Indicates that the correlations were significant at ($p \leq 0.05$), r=correlation coefficient

4.5.7 Effect of cropping practices in relation to potato cyst nematode population density

Effect of intercropping, mono-cropping and relay cropping on potato cyst nematode population density

Potato farmers practicing mono-cropping were 47.7 % while intercropping and relay cropping was practiced by 46.2 % and 4 % of the farmers, respectively. The lowest number of cysts 238, 287.5 and 49 cysts/ 300 g soil were recovered from intercropped potato farms in UH3, UH2 and LH4 AEZs respectively. On the other hand, the highest cysts counts were recovered from mono-cropped farms in UH3, UH2 and LH4 AEZ having 285, 322.0 and 81 cysts/ 300 g soil, respectively (Table 4.5).

Effect of crop rotation on potato cyst nematode population density

The frequency of farms where crop rotation was practiced was 93.8 %. The PCN population density across the three AEZs where farmers rotated their potato crops was significantly ($p \leq 0.05$) low in UH3-92.1, UH2- 249.8, LH4-28.2 cysts/ 300 g compared to UH3- 242.5, UH2-1073 LH4- 377.3 cyst/ 300 g where crop rotation was not practiced (Table 4.6).

Eight different crops namely maize, peas, cabbages, beans, carrots, snow peas, kales and oats were grown in rotation with potato across the three AEZs (Figure 4.3). In UH3 and UH2, maize was the most commonly used rotational crop in 36.9 and 37 % of the farms, respectively. In LH4, majority of farmers (31.5 %) used peas as their rotational crop (Figure 4.3).

Table 4.5. Effect of cropping system on potato cyst nematode population density in three agro-ecological zones in Nyandarua county, Kenya

Cropping System	Agro ecological zone						Percentage infested samples
	UH3		UH2		LH4		
	Mean No. of PCN/300 g soil	Standard Deviation	Mean No. of PCN/300 g soil	Standard Deviation	Mean No. of PCN/300 g soil	Standard Deviation	
Intercrop	172	304.2	238	287.5	49	88.4	46.2
Mono crop	225	220.5	285	322.0	81	216.2	47.7
Relay crop	344	369.0	-	-	-	-	6.2

Table 4.6. Effect of rotated and non-rotated potato crop on potato cyst nematode population density in three agro-ecological zones in Nyandarua county, Kenya

Practicing crop rotation	No. of farmers	Percentage farmers	Agro ecological zone						Combined AEZs	
			UH3		UH2		LH4			
			Mean PCN/300 g soil	Standard Deviation	Mean PCN/300 g soil	Standard Deviation	Mean PCN/300g soil	Standard Deviation	Mean PCN/300 g soil	Standard Deviation
No	4	6.2	242.5		1037		377.3	336.9	508.5	407.5
Yes	61	93.8	92.1	241.7	249.8	308	28.2	73.2	15.5.6	246.6
Total	65	100	203.7	236.7	289.2	347.7	63.1	149.4	186.7	267.8

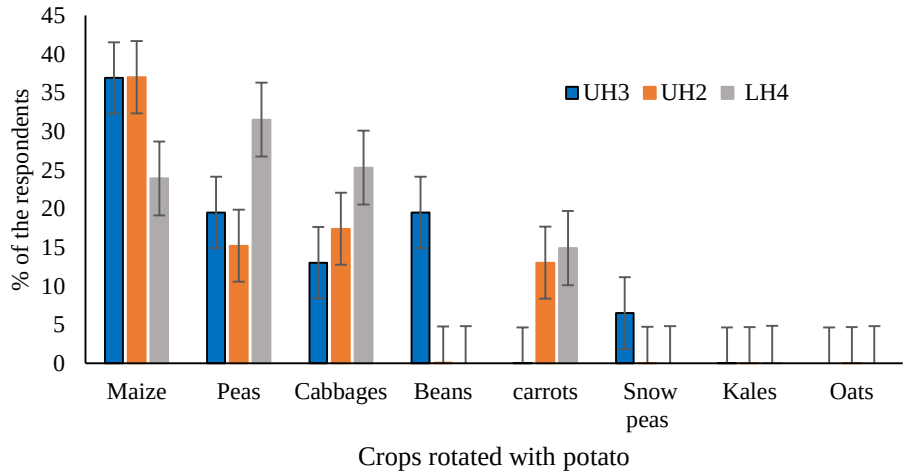

Figure 4.3. Crops commonly rotated with potato by farmers (% respondents) in three agroecological zones in Nyandarua, county

Effect of length of rotations on potato cyst nematode population density

The rotational lengths of the 65 sampled sites were determined and the most common rotation length was that of one potato crop after two seasons where 36.9 % farms were recorded (Figure 4.4). Only 6.2 % farms did not practice crop rotation. The longest rotational period was after 6 seasons where only one farm was registered (Figure 4.4).

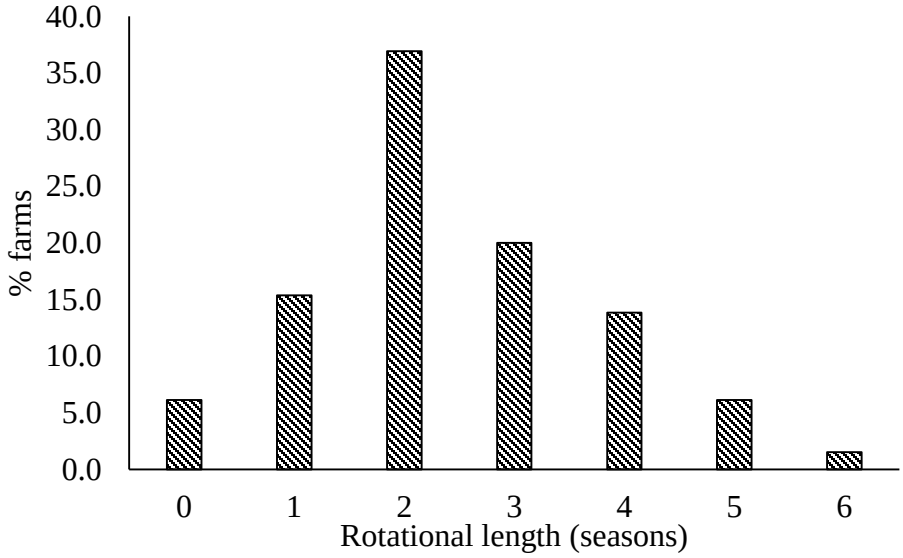

Figure 4.4. Crop rotation length, in seasons, between successive potato crops in the same plot in Nyandarua county

Effect of potato crop cycles per year in relation to potato cyst nematode population density

Farms where only one potato crop was planted in a year had the least cyst count of 38.5 cyst/300 g. A significantly ($p \leq 0.05$) higher cyst count of 302 cysts/ 300 g of soil was observed in farms where four potato crop cycles were grown per year compared to farms growing fewer potato crops per year (Figure 4.5).

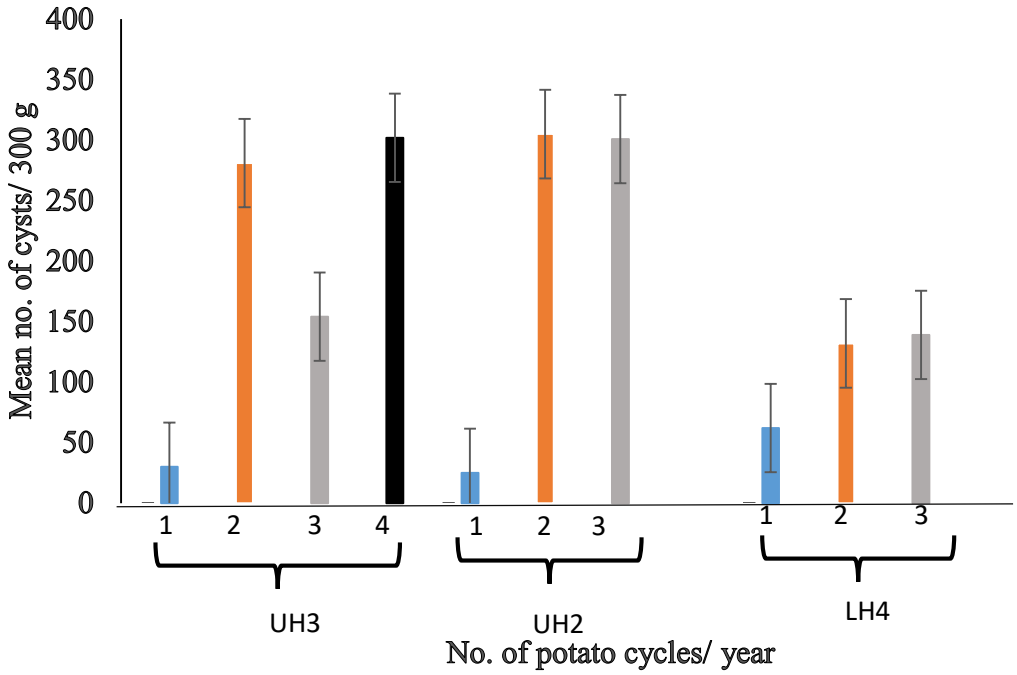

Figure 4.5. Number of potato crops grown in the same field per year in relation to mean population density (PCN cysts/300 g soil) of potato cyst nematode detected in potato fields in three AEZs, Nyandarua county, Kenya.

Relationship between seed source and PCN population density

The study showed that 92.3 % of the farmers used non-certified seed, while 7.7 % used certified seed. The cyst count /300 g soil was significantly ($p \leq 0.05$) higher in farms that did not use certified seed with 200.7 cysts /300 g soil, compared to an average of 60.2 cysts /300 g soil in farms that did use certified seed (Table 4.7).

Cultivars grown in relation to PCN population density

A total of five cultivars (Shangi, Dutch Robjin, Kenya Mpya, Tigoni and Mubiru) were recorded during the survey (Table 4.8). Majority (98. 5%) of the sampled sites were cropped with Shangi and had significantly ($P \leq 0.05$) higher mean cyst count that ranged from 174.3 to 463.1 cysts/ 300 g soil compared to varieties Kenya Mpya, Tigoni, Mubiru and Dutch Robjin which had significantly lower cyst counts in all the AEZs (Table 4.8).

Effect of diseases in relation to potato cyst nematode population density

Late blight was the most common disease observed, and potato cyst nematode was the most common pest in all AEZs (Table 4.8). The results showed that 96.9% of potato farms had late blight followed by PCN (95.4%), bacterial wilt (76.9%) and viral diseases (18.5 %). Across sites, the highest number of potato cyst nematodes (264.4 cysts /300 g soil) occurred in fields which had viruses (Table 4.9).

Table 4.7. Effect of seed source on the mean population density (cysts/300 g) of potato cyst nematode in three agro-ecological zones, Nyandarua county, Kenya

Seed source	Agro ecological zone													
	UH3					UH2			LH4			Total		
	No of farms	Percentage No. of farms	No. of farms	Mean number of cysts/ 300 g soil	Standard Deviation	No. of farms	Mean number of cysts/ 300 g soil	Standard Deviation	No. of farms	Mean number of cysts/ 300 g soil	Standard Deviation	No of farms	Mean number of cysts/ 300 g soil	Standard Deviation
Non certified seed	60	92.31	22	69.6	251.7	20	303.4	356.6	18	247.3	156.5	60	200.7	280.7
Certified seed	5	7.69	3	4.3	80.5	1	26.2	46.2	2	29.7	2.5	5	60.2	83.0

Table 4.8. Influence of potato cultivars on potato cyst nematode density in three agro-ecological zones, Nyandarua county, Kenya

Potato variety grown	Agro ecological zone										Total		
	UH3			UH2			LH4						
	No of farms	Mean of cysts/ 300 g soil	Standard Deviation	No. of farms	Mean of cysts/ 300 g soil	Standard Deviation	No. of farms	Mean of cysts/ 300 g soil	Standard Deviation	No. of farms	% number of farms	Mean of cysts/ 300 g soil	Standard Deviation
Shangi	24	174.3	89.5	20	289.2	347.7	20	463.1	249.4	64	98.5	175.4	253.8
Dutch Robjin	0	0	0	1	5.5		0	0	0	1	1.5	5.5	
Kenya Mpya	2	61.8	41.2	0	0	0	0	0	0	2	3.0	61.8.0	41.2
Tigoni	0	0	0	1	32.5		0	0	0	1	1.5	32.5	
Mubiru	0	3.5	0	1	107.0		0	0	0	1	1.5	107	0

Table 4.9. Potato pests and diseases present across the three agro-ecological zones, Nyandarua county, Kenya

Pest and diseases	UH3			UH2			LH4			Combined AEZs		
	No. of infected (farms) by disease/pests	% infestation	PCN population density at 300 g	No. of infested samples (farms)	% infestation	PCN population density at 300 g	No. of infested samples (farms)	% infestation	PCN population density at 300 g	No of infested samples (farms)	% infestation	PCN population density at 300 g
Bacterial wilt	21	84.0	44.9	14	70.0	150.6	15	75.0	302.0	50	76.9%	151.7
Rhizoctonia	7	35	4.7			14.0	8	40	165.3			77.4
Late blight	23	92.0	63.1	20	100.0	2892	20	100.0	163.9	63	96.9%	172.1
Viruses (PLRV)	4	16	3.3	5	25.0	402.7	3	15.0	277.9	12	18.5%	264.4
*PCN	25	100	66.4	17	68.0	289.2	20	100.0	254.4	62	95.4	202.1

*This was established after cysts extraction from samples collected. The other diseases were observed during the farm interview.
PLRV: Potato leaf roll virus

4.5.8 Sources and spread of potato cyst nematodes

Cleaning and disinfestation of tools, footwear and planting seed

All the farmers did not clean nor disinfect their agricultural tools (hoe) and seed potatoes. Only about 10% of the farmers reported that they sometimes cleaned their footwear after use but did not disinfect them. None of the farmers was aware that cleaning and disinfecting of hoe, footwear and seed potatoes could minimize the spread of pests.

Number of PCN cysts and eggs recovered on seed, hoe and footwear

The number of cysts recovered from soil adhering to hoe, seed and footwear in UH3 and LH4 were significantly ($p \leq 0.05$) higher than that of UH2 agro ecological zone (Figure 4.6 and 4.7). A significantly ($p \leq 0.05$) lower number of cysts were recovered from UH2 soils having 1.8, 2.6, 2.8 cysts/50 g from footwear, farm tools and seed respectively (Figure 4.6). The number of cysts adhering onto hoe, footwear and seed ranged from 2.6 to 13.7, 1.8 to 8.8, and 2.8 to 12.9 cysts/50 g soil respectively. A significantly ($p \leq 0.05$) higher number of viable eggs 120.4 /cyst were recovered from soil adhering to seed in UH3 compared to UH2 and LH4. While in UH2, significantly low numbers of PCN eggs were recovered from footwear, farm tools and seed with 38.2, 39.4 and 54.9 viable eggs/cyst respectively (Figure 4.7).

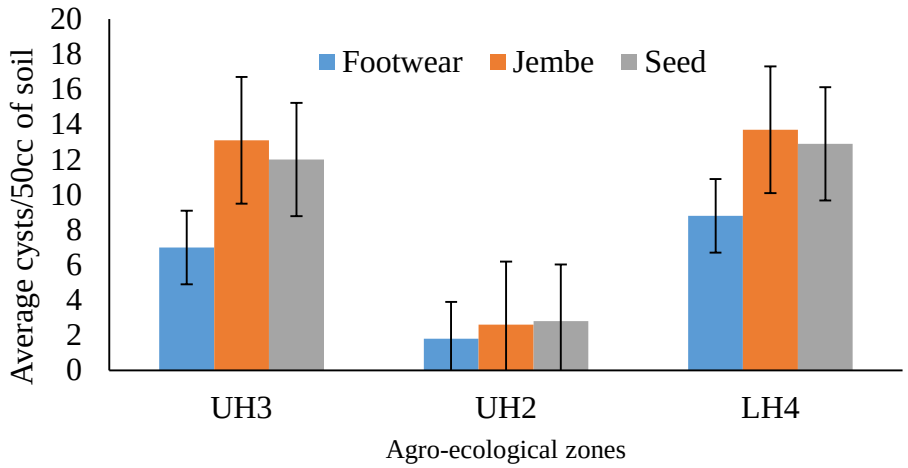

Figure 4.6: Mean number of cysts recovered from 50 g of soil from footwear, hoe and seed potato in the three agro ecological zone in Nyandarua county

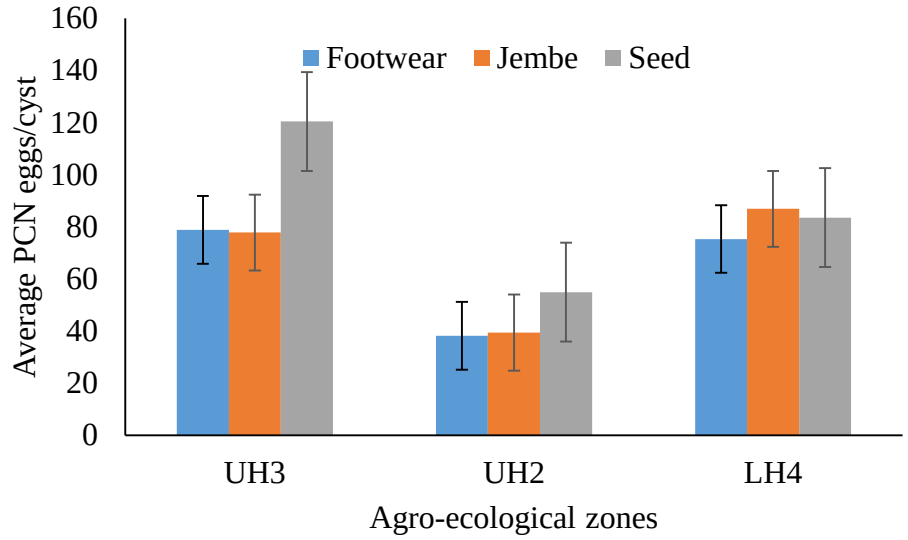

Figure 4.7. Mean number of viable PCN eggs recovered from soil attached to footwear, hoe and seed potato in the three agro ecological zone in Nyandarua county

4.6 Discussion

This study has revealed that potato cyst nematodes are widespread in Nyandarua county with over 95 % of the farms being infested. The high prevalence of PCN gives an indication of the rapid spread of the pest given that the pest was first reported to be present in Kenya about five years ago. The findings are consistent with previous studies that reported high prevalence (90 %) and high levels of infestation of PCN above economic thresholds in the potato growing counties in Kenya (Haukeland, 2016; Mburu *et al.*, 2020).

According to the findings of this study, the surveyed sites had low temperature of less than 25^{o}C. Such temperatures are suitable for reproduction of PCN because they prevent desiccation and dryness in the soil. They are also suitable for maintaining a thin film of water around the juveniles for ease of mobility and therefore access to food (Skelsey *et al.*, 2018). This finding corresponds with a prior research where high percentage hatch of *G. rostochiensis* occurred at 10 to 25^{o}C (Kaczmarek *et al.*, 2014). The ambient temperatures recorded in this study may have favoured *G. rostochiensis* reproduction as they are within the reproductive range for the species.

The present study shows that annual rainfall >1000 mm is more favourable to high PCN densities compared to annual rainfall of <1000mm. This is probably because of runoff which is a source of PCN spread and occurs with high rainfall. This hypothesis is supported by the work of Yusianto *et al.*, (2020) who observed PCN spread occurs in a number of ways including rainfall runoff. Rainfall has also been reported to have a significant impact on PCN population densities as well as the safety of their soft bodies by preventing desiccation (Nisa *et al.*, 2021).

The high altitude (>2300 masl) of the study site positively influenced the PCN population density. The high altitude could have resulted in cool temperatures and high rainfall that favours PCN reproduction. This is consistent with Yusianto *et al.* (2020) who noted that at an altitude of 2000 masl, PCN had the most serious attack and high density. A high PCN population density was recovered in sandy, clay loam and loam soils having above 40 % sand, >20 silt and 28-37 % clay. Sandy clay soils having 48 % sand, 16 % silt and 36 % clay and registered the lowest number of PCN. Soil texture is associated aeration, water holding capacity, root system and distribution of species in the soil (Quist *et al.*, 2019). This could explain the high spread and population density

of PCN observed in this study's sandy, clay loam and loam soils. According to Prot and Van Gundy (1981) soil type is important in influencing nematode distribution and density, with both clay and silt soils inhibiting *Meloidogyne incognita* mobility thus reducing their population.

Among the surveyed farms, no potato grower was found to be using nematicides or practicing any control measures thus increasing the PCN build up. Additionally, during the interviews with the farmers about their awareness of PCN, majority stated that they were not aware of PCN in potato and were more concerned with late blight and bacterial wilt. This could have been due to failure to recognize visual symptoms. Mburu *et al.* (2020) pointed out that, PCN have unclear symptoms making the pest to be overlooked by majority of potato growers in Kenya. Many farmers were also not aware of integrated approaches of managing the pest, indicating a gap in farmer knowledge.

The current study found cysts in seed, footwear and machinery/farm tools although they were very few. The few cysts could potentially form an invasive population. According to Banks *et al.* (2012), one cyst from a gram of soil can reproduce 30 cysts in a season. Furthermore, during the study it was observed that majority of farmers in Nyandarua county rely on non-certified seed sources such as their own farm saved seed, markets and neighbours. According to Kinyua *et al.,* (2001) non-certified are infested with pests and diseases, therefore accelerate spread of seed borne diseases and pests. It is therefore speculated that seed, hoe and footwear could be the primary sources of PCN spread in Kenya. Moreover, nematodes do not move long distances on their own and are therefore unable to spread from one field to the other on their own. In previous studies, seed and farm equipment have been blamed for the rapid spread of PCN, through soil attached on the seed potatoes and the farm equipment's from infested fields (Briar *et al.,* 2016: Mburu *et al.*, 2020). Many farmers commonly hire casual labour during certain parts of the production season. Often times the hired labourers come with their tools which are not disinfected before use. This practice is also likely to have contributed to rapid spread of PCN.

It was noted that variety Shangi was grown by almost all farmers. According to Mburu *et al.* (2020), variety Shangi is susceptible to PCN. It is therefore possible to infer that the wide spread planting of the susceptible variety Shangi in Kenya has accelerated PCN infestation in potato production fields, which is accelerated by relay cultivation of the variety. Previous studies indicate

that there is significant relation between PCN and use of resistant and non-resistant cultivars whereby cultivars resistant to PCN would possibly reduce PCN density (Grubisic *et al.*, 2007). However, it is a challenge in Kenya to deploy the resistance aspect of PCN when the greatest appeal is the market choice of the variety. Additionally, many potato cultivars in Kenya are of unknown PCN resistance and those having partial resistance to PCN (KEPHIS, 2020) are limited in availability since they have breeders rights. This indicates a serious problem in subsequent years due to intensive cultivation of a susceptible variety (Shangi) and lack of partially resistant or resistant varieties in the Kenyan market.

The findings of this study showed that farms that intercropped and rotated their potato crops recorded a lower PCN population density compared to farms that mono and relay cropped potato crops. The lower PCN population densities in the intercrop and rotational potato production systems could be attributed to the crops used which were non-hosts. These included peas, cabbages, beans, carrots, snow peas, kales and oats. According to Briar (2016), planting non-host crops in rotation would remove the food supply for plant parasitic nematodes to survive, thus preventing the colonization and establishment of PCN consequently causing their population to drop below the damaging levels. Moreover, different crop species planted as intercrop or rotational crops can give antagonistic effect on nematode populations while increasing crop yield by reducing crop pests or providing other agronomical benefits such as maintaining soil fertility, which leads to increased crop yield (Bairwa *et al.*, 2017).

According to this survey, majority of the farmers had a short rotational duration of one in two seasons or lower. These practices partly explain the high number of PCN reported in this study. Trudgill (2014) reported similar findings, observing that crop rotation reduced *G. pallida* eggs per gram of soil by 26.2 % per year in commercial potato crops. Skelsey *et al.* (2018) recommend a rotation with a non-host crop for PCN, while a minimum of one crop every six years for seed production in Scotland was effective management strategy in reducing PCN risk.

CHAPTER 5: RELATIVE YIELD LOSSES OF POTATO IN FIELDS INFESTED WITH POTATO CYST NEMATODES IN NYANDARUA COUNTY, KENYA

5.1 Abstract

Despite the potato cyst nematodes (PCN) infestations in Kenya, information on associated yield losses is limited. This study was carried out to determine relative yield losses associated with damage by PCN. Three on-farm sites in Nyandarua county with varying PCN densities (low-1-9, moderate-10-39, and high 40-80 cysts in 100g/soil) were chosen for the study during the long and short rains seasons, 2021. Treatments consisted of three potato cultivars namely Desiree and Shangi that are susceptible as well as Manitou which is partially resistant. The four PCN control products that were tested were fluopyram (Velum), oxamyl (Vydate), azandirachtin (commercial neem extract Achook) and the fungi *Paecilomyces lilacinus* (Mytech). The experiments were set up in a randomized complete block design, in a split plot arrangement, with varieties as the main plots, and PCN control products including the control as the sub-plots. Treatments were replicated three times and the entire experiment repeated once. The reproductive index of the nematodes ranged from 0.17 to 2.39 during the (cool) long rains and from 0 to 4.13 during the (warm) short rainy season. During the long rainy season, relative yield loss due to PCN infection ranged from 6.3 % to 80.5 %, while during the short rainy season, it ranged from 5.5 % to 73.3 %. The magnitude of yield losses was dependent on susceptibility of the variety, the initial PCN population density and the PCN control product used. This study has demonstrated that PCN are already causing significant yield and economic losses especially in heavily and moderately infested fields. It was shown that oxamyl was the most effective nematicide in reducing PCN numbers and yield losses. The yield losses demonstrated in this study necessitate the need to institute urgent measures to combat the pest if the country's food security targets are to be realized. Farmers should be advised to take appropriate measures to prevent spread and build-up of the nematodes.

5.2 Introduction

In Kenya, potato (*Solanum tuberosum* L.) which ranks second in value after maize, is important for ensuring national food security and poverty alleviation (MoALF, 2021). The crop is grown for food and cash by both small and large-scale farmers. Potato production is estimated to be 2,107,824

tons, valued at about KSh.50 billion (416,000,000 USD) at farm gate prices and is produced on an estimated area of 214,600 ha year^{-1} (FAOSTAT, 2021; MoALF, 2021).

Potato cyst nematodes (PCN) (*Globodera rostochiensis*) which were first detected in Kenya in 2014, are among the pests that limit potato production and quality (Mwangi, *et al.*, 2015). Over 80 % of the farms in Kenya are infested with PCN (Haukeland, 2016). Potato cyst nematode spread and build-up is increasing due to lack of awareness by farmers, little use of crop rotation and few resistant cultivars available, thus posing a threat to potato production (Chapter 4, Mburu *et al.,* 2020).

Nematodes are soil borne pests, which are spread by rain water, floods, animals, farm implements, and footwear (Chapter 4, Contina *et al.,* 2018; EPPO, 2017). Human activities that are associated with movement of potato tubers during harvesting and trade are a major contributor to spread of PCN (Chapter 3, Mark *et al.,* 2019). Potato cyst nematodes are sedentary endo-parasites which form modified feeding sites known as syncytia at the vascular region of root cells (Hajihassani *et al.,* 2013). The pest has a high reproductive potential since the tiny cysts contain 200 to 600 eggs. Infestation by PCN results into symptoms on the vegetative parts and below ground which are not very distinct. Because of the nematode feeding activity, which disrupts water and nutrient uptake, the above ground part of the plant shows poor growth, yellowing, stunting, and wilting (EPPO, 2017). The root system of infested plants may be reduced in the soil, with mature females present on the root surface and small sized tubers observed (Hajihassani *et al.,* 2013). The cysts are hardy and long-lived 20 to 30 years without a host, even under unfavourable conditions, thus making it difficult to control once a field is infested with PCN (Niere and Karuri, 2018). Under heavy infestation, yield losses caused by PCN could reach 80% if left uncontrolled (Deliopoulos *et al.,* 2010; Haukeland, 2016; Hodda and Cook, 2009; Mburu *et al.,* 2020). Many factors influence yield loss, including nematode population density, potato cultivar, and management practices in place (Djebroune *et al.,* 2020).

Use of certified seeds, cultural methods, crop rotation, cultivation of resistant varieties, nematicides, inter cropping, and trap crops are some of the alternative methods for pest management (Lopez-Lima *et al.,* 2013). Farmers all over the world have relied on commercial nematicides in controlling plant parasitic nematodes (Renco and Kovacik, 2015). Overuse of these

nematicides may lead to development of pathogen resistance, and some are environmentally hazardous.

Host resistance is the most pragmatic method of managing PCN with the added advantage of being environmentally friendly (Faggian *et al.,* 2012). It also reduces nematicide costs and the associated costs of application. Nevertheless, when these methods are applied singly, they are less effective against PCN. Thus, as part of integrated pest management (IPM) the employment of cultural, chemical, biological and use of resistance varieties as management strategies is advised in farms with PCN infestations. However, there is little data on the effects of integrated PCN management through host resistance and PCN control products in farmers' fields in Kenya. As a result, a study to determine the magnitude of relative potato yield losses due to PCN at varying infestation levels in Nyandarua county was conducted.

5.3 Materials and methods

5.3.1 Description of the study sites

Three on-farm sites were chosen based on Jones' (1970) modified classification of PCN densities as low-1-9, moderate-10-39, and high-40-80 cysts in 100g/soil in Nyandarua county during the long and short rains seasons, 2021. The PCN population densities were established through a survey carried out at the sites (Chapter 4). The first and repeat experiments were conducted in 2021 during the long and short rainy seasons, respectively. Table 5.1 shows the characteristics of the experimental sites.

5.3.2 Treatments and experimental design

Three potato varieties were used in the study. Potato cultivar Shangi was selected as the most commonly grown variety while Desiree and Manitou were selected as susceptible and resistant cultivars, respectively (Mburu *et al.*, 2020; NPCK, 2019, Nivap, 2011; EPPO, 2006). The experiment had five treatments: two synthetic nematicides fluopyram (Velum) and oxamyl (Vydate), Azadirachtin a commercial neem based nematicide (Achook), a bio-control agent, *Paecilomyces lilacinus* (Mytech), and a control in which no PCN control product was applied. The treatments were applied according to the guidelines from the manufacturers.

Table 5.1. Characteristics of the experimental sites used in assessment of relative yield loss due to potato cyst nematodes in Nyandarua county

Site	Latitude	Longitude	Altitude (M asl)	AEZ	*PCN level of infestation/ Densities	Cyst densities in 100 g soil
Ol Joro Orok	0° 4' 0" S	36° 22' 0" E	2750	UH2	Low	1-9
Kinangop	0°43'50.8"S	36°39'32.6"E	2390	UH3	Moderate	10-39
Kipipiri	0.4379° S	36.5013° E	1840	LH4	High	40-80

*Modified from Jones (1970)

Experiments were set up in a randomized complete block design, in a split plot arrangement, with varieties in the main plots, and PCN control products including the control in the sub-plot. Treatments were replicated three times and plots measured 2.5 m x 3 m with four rows of ten hills each. Well sprouted medium sized (35-45 mm) certified seeds were planted at a spacing of 30 by 75 cm. All the agronomic practices for potato production, except spraying of insecticides, were followed. Di-ammonium phosphate fertilizer (18:46:0) fertilizer was applied at planting at the rate of 500 kg ha^{-1}.

5.3.3 Data collection

Pest parameters

Data on number of cysts in the soil were collected at planting (initial population-Pi) and at harvest, 90 days after planting (population final-Pf).

Yield data

At harvest, two inner rows, excluding the first and last hill in each row (guard plants), were used to calculate yield, which was expressed as t ha^{-1}.

5.3.4 Data analysis

Shapiro-Wilk test was used to determine normality of the cyst and yield data (Shapiro and Wilk, 1965). Non-normal data were transformed using square root (x +1), where x is the original value of the variable. The PCN cyst reproduction index was expressed as RI = (Pf/Pi) according to Van Den Berg and Rossing (2005), where: Pi is the initial population density of potato cyst nematodes, Pf is the final population density. The relative percent yield loss for each treatment was calculated using the method described by (Robert and James, (1991). The formula was as follows:

$$\text{Relative yield loss } (\%) = \frac{\text{Ybt} - \text{Yot}}{\text{Ybt}} \text{X}100$$

Where Ybt denotes the mean total yield of the best experimental treatment in the experiment and, Yot: denotes the mean yield of other experimental treatments.

Analysis of variance (ANOVA) was performed using SAS 8.2 Edition, and means compared using Fischer's protected least significant differences (LSD) test at ($p \leq 0.05$). Correlation analysis was used to determine the relationship between the final number of cysts, the PCN reproductive index, and tuber yield.

5.4 Results

5.4.1 Initial cyst population density

The initial cyst population counts taken from the experimental sites confirmed that the sites fall into the three PCN densities groupings that were rated as low, moderate and high PCN density. Table 5.2 shows that during the long rains season, the initial cyst population varied from 6 - 10, 24 -33, and 52 -84 cyst/100 g soils in the low, moderate, and high PCN density sites, respectively. During the short rains season, PCN density varied from 5 - 9, 23 - 35 and 40 -55 cysts/100g soil in the respective sites indicating a similar trend (Table 5.3).

Table 5.2. Effect of potato cyst nematode (PCN) control products, infestation levels and variety on the nematode population dynamics during the long rains season 2021

Level of PCN infestation and treatments	Susceptible variety (Desiree)				Susceptible variety (Shangi)				Resistant variety (Manitou)			
	Cyst/300 g soil				Cyst/300 g soil				Cyst/300 g soil			
	Initial	Final	% change	RI	Initial	Final	% change	RI	Initial	Final	% change	RI
Low PCN infestation												
Oxamyl	9	3(1.8)	-66.6	0.33	8	2(1.9)	-75.0	0.25	6	1(1.0)	-83.3	0.17
Fluopyram	7	4(2.0)	-42.9	0.56	8	3(1.7)	-62.5	0.34	9	3(1.6)	-66.7	0.28
Azadirachtin	8	4(2.1)	-50.0	0.55	9	4(2.1)	-55.6	0.50	9	2(1.4)	-77.8	0.24
P. lilacinus	7	5(2.2)	-28.6	0.72	10	5(2.3)	-50.0	0.55	8	3(1.6)	-62.5	0.32
Control	8	17(4.1)	112.5	2.39	8	17(4.1)	112.5	2.09	10	16(3.9)	60.0	1.62
Moderate PCN Infestation							-					
Oxamyl	31	9(1.8)	-70.9	0.29	30	16(3.8)	46.7	0.48	27	10(3.0)	-63.0	0.35
Fluopyram	30	21(4.5)	-30.0	0.69	32	17(4.2)	-46.9	0.54	29	12(3.4)	-58.6	0.42
Azadirachtin	31	21(4.6)	-32.3	0.69	26	16(4.0)	-38.5	0.62	33	19(4.3)	-42.4	0.56
P. lilacinus	27	22(4.7)	-18.5	0.82	24	13(3.6)	-45.8	0.56	28	15(3.8)	-46.4	0.53
Control	27	39(6.2)	44.4	1.51	31	58(7.6)	87.1	1.88	29	39(6.3)	34.5	1.44
High PCN infestation												
Oxamyl	52	32(4.9)	-38.4	0.61	68	41(6.1)	-39.7	0.60	63	30(5.5)	-52.4	0.47
Fluopyram	64	42(6.4)	-34.4	0.65	84	54(7.3)	-35.7	0.64	65	31(5.5)	-52.3	0.48
Azadirachtin	65	49(6.9)	-24.6	0.74	62	43(6.5)	-30.6	0.69	60	35(5.9)	-41.7	0.58
P. lilacinus	52	41(6.3)	-21.2	0.78	62	43(6.6)	-30.6	0.70	60	36(6.0)	-40.0	0.59
Control	61	93(9.6)	52.5	1.53	65	98(9.9)	50.8	1.49	61	70(8.3)	14.8	1.15

Data in parenthesis are transformed data; Data are means of three replications *P. lilacinus* stands for *Paecilomyces lilacinus*.

For initial number of cysts: CV (%) =21, LSD $_{site}$=2.9*

For final cyst population: CV (%) =32.1%, LSD $_{PCN\ control\ product}$=4.6*; LSD $_{variety}$=3.5*; LSD $_{site}$=3.5*, LSD $_{PCN\ control\ product\ x\ variety}$=7.9^{NS}; LSD $_{PCN\ control\ product\ x\ site}$=7.9*, LSD $_{variety\ x\ site}$=6.1*; PCN control product x variety x site =13.7^{NS}

For Reproduction Index (RI): CV (%) =29.4%, LSD $_{PCN\ control\ product}$=0.124*; LSD $_{variety}$=0.096*; LSD $_{site}$=0.096^{NS}; LSD $_{PCN\ control\ product\ x\ variety}$=0.215^{NS}; LSD $_{PCN\ control\ product\ x\ site}$=0.215*; LSD variety x site=0.166^{NS}; PCN control product x variety x site =0.372^{NS}NS-Not significant; * significant at ($p \leq 0.05$)

Table 5.3. Effect of potato cyst nematode (PCN) control products, infestation levels and variety on the nematode population dynamics during the short rains season 2021

Level of PCN infestation And treatments	Susceptible variety (Desiree)				Susceptible variety (Shangi)				Resistant variety (Manitou)			
	Cyst/300 g soil				Cyst/300 g soil				Cyst/300 g soil			
	Initial	Final	% change	RI	Initial	Final	% change	RI	Initial	Final	% change	RI
Low PCN infestation												
Oxamyl	8	2(1.7)	-75.0	0.25	7	0(1.2)	-100	0.07	6	0(1.0)	-100.0	0.00
Fluopyram	8	3(2.0)	-62.5	0.39	6	1(1.4)	-83.3	0.15	6	0(1.0)	-100.0	0.00
Azadirachtin	6	2(1.7)	-66.7	0.32	7	2(1.8)	-71.4	0.30	7	1(1.5)	-85.7	0.16
P. lilacinus	5	3(1.9)	-40.0	0.48	8	3(1.9)	-62.5	0.33	7	2(1.6)	-71.4	0.26
Control	8	31(5.6)	287.5	4.13	9	33(6.1)	266.0	5.73	7	15(4.0)	114.3	2.32
Moderate PCN infestation												
Oxamyl	29	8(3.2)	-72.4	0.27	29	4(2.0)	-86.2	0.14	30	6(2.7)	-80.0	0.21
Fluopyram	35	29(5.2)	17.1	0.79	28	6(2.6)	-78.6	0.21	31	10(3.3)	-67.7	0.33
Azadirachtin	32	15(3.8)	-53.1	0.43	23	7(2.8)	-69.6	0.31	28	10(3.3)	-64.3	0.37
P. lilacinus	31	15(3.9)	-51.6	0.46	30	12(3.5)	-60.0	0.38	27	10(3.3)	-63.0	0.37
Control	35	67(8.3)	91.4	1.97	31	63(8.0)	103.2	2.04	34	29(5.4)	-14.7	0.85
High infestation												
Oxamyl	51	24(4.7)	-52.9	0.42	48	15(3.9)	-68.8	0.30	41	8(3.0)	-80.5	0.20
Fluopyram	49	33(5.8)	-32.7	0.66	47	20(4.5)	-57.4	0.42	50	8(3.1)	-84.0	0.17
Azadirachtin	47	31(5.6)	-34.0	0.67	40	22(4.8)	-45.0	0.56	47	18(4.2)	-61.7	0.36
P. lilacinus	40	21(4.6)	-47.5	0.55	46	28(5.3)	-39.1	0.59	55	25(5.1)	-54.5	0.47
Control	44	75(8.7)	70.5	1.82	47	76(8.8)	61.7	1.64	39	37(6.1)	-5.1	1.00

Data in parenthesis are transformed data; Data are means of three replications: For initial number of cysts: CV (%) =29.5, LSD_{site}=3.4*

For final cyst population: CV (%) =22.2%, $LSD_{PCN\ control\ product}$=0.47*; $LSD_{variety}$=0.36*; LSD_{site}=0.36*, $LSD_{PCN\ control\ product\ x\ variety}$=0.81*; $LSD_{PCN\ control\ product\ x\ site}$=$0.81^{NS}$, $LSD_{variety\ x\ site}$=0.63^{NS}; $LSD_{PCN\ control\ product\ x\ variety\ x\ site}$ =1.41^{NS}

For Reproduction Index (RI): CV (%) =25.9%, $LSD_{PCN\ control\ product}$=0.10*; $LSD_{variety}$=0.08*; LSD_{site}=0.08^{NS}; $LSD_{PCN\ control\ product\ x\ variety}$=0.18*; $LSD_{PCN\ control\ product\ x\ site}$=0.18*; $LSD_{variety\ x\ site}$=0.14^{NS}; $LSD_{PCN\ control\ product\ x\ variety\ x\ site}$ =0.31^{NS}NS-Not significant; * significant at $(p \leq 0.05)$

5.4.2 Effect of nematode control products and variety on cyst dynamics

Final cyst population density

The effects of PCN control product, site and variety on the final number of cysts were significantly different ($p \leq 0.05$) in both seasons. The interaction between PCN control product x variety was significant. However, the interactions between PCN control product x site and that between PCN control product x variety x site were not significant (Tables 5.2 and 5.3).

All PCN control products led to a significant decrease in final PCN counts compared to the untreated control. For example, during the long rains season 2021, the untreated plot of Desiree grown in a heavily PCN infested soil had a final PCN count of 75 cysts/100 g soil, while cyst counts in plots where PCN control products were applied varied from 21-33 cysts/ 100 g soil (Table 5.2). A similar observation was made during the short rains season where potato cultivar Manitou grown in a heavily infested and untreated soil had a final cyst count of 37 compared to 8-25 cysts /100 g soil in plots to which control products were applied (Table 5.3).

In general, the final PCN cyst counts tended to increase with increasing initial PCN population density regardless of the PCN control product applied or the variety grown during both seasons. For example, during the long rains season, Desiree in a low PCN density site and treated with oxamyl had a final PCN count of 3 cysts per 100 g soil. However, when grown in a medium PCN density site, the final PCN population was 9 cysts per 100 g soil while at the high PCN density site the final PCN count was 32cysts per 100 g soil (Table 5.2). A similar trend was observed during the short rains season. Among the PCN control products, oxamyl had the highest PCN reduction ranging from 38.4 to 100% (Table 5.2 and 5.3).

Cysts reproductive index

All the PCN control products used in this study significantly inhibited cysts reproduction compared to the untreated control. Variety Manitou significantly limited the cysts reproduction relative to the susceptible varieties Desiree and Shangi (Table 5.2 and 5.3). During the long rains season the RI ranged from 0.17 for variety Manitou planted in a low PCN population density site and sprayed with oxamyl to 1.44 planted in moderately infested site. Variety Shangi recorded the highest

reproductive index of RI=5.73 at the moderately infested site (Table 5.3). When PCN control products were used in all three sites, the reproductive indexes of Shangi and Desiree varieties were not significantly different ($p \leq 0.05$).

5.4.3 Effects of nematicide application and variety on yield

5.4.3.1 Number of tubers per plant

During both seasons, the number of tubers per plant was significantly influenced by variety but not by site or PCN control product. The interactions between variety, site and PCN control product were also not significant (Tables 5.4 and 5.5). The range in the number of tubers per plant varied from 5.9 (Desiree, moderately PCN infested site and without any PCN control product) to 12.7 (Shangi, moderately PCN infested site and treated with Azadirachtin (Achook) and from 5.7 (variety Desiree planted in a low PCN infested site and without any PCN control product applied to it) to 12.6 (variety Shangi planted in a heavy PCN infested site and treated with fluopyram) during the long rains and short rains seasons, respectively. Variety Desiree had significantly lower number of tubers compared to either variety Shangi or Manitou regardless of the PCN control product applied or initial level of PCN infestation during both seasons.

5.4.3.2 Mean tuber weight

During both seasons, the effects of PCN control product, variety, and site on mean tuber weight were significant. The interaction between PCN control product and variety on mean tuber weight was only significant during the long rains season but not during the short rains season. The effects of the interactions of PCN control product x site, variety x site and PCN control product x variety x site on mean tuber weight were not significant during both seasons (Tables 5.4 and 5.5). Mean tuber weights varied from 13.6 (variety Desiree planted in a heavily PCN infested site and without any nematicide application) to 86.9 g/tuber (variety Manitou planted in a low PCN infested site and treated with oxamyl) and from 13.9 g/tuber (Variety Desiree planted in a heavy PCN infested field and without nematicide application) to 77.9 g/tuber (Variety Manitou grown in a heavily PCN infested site and sprayed with oxamyl) during the long rains 2021 and short rain 2021 seasons, respectively (Table 5.4 and 5.5).

Table 5.4. Effect of potato cyst nematode (PCN) control products, infestation levels and variety on number of tubers per plant, mean tuber weight and marketable yields during the long rains season 2021

Level of PCN infestation and treatments	Susceptible variety (Desiree)				Susceptible variety (Shangi)				Resistant variety (Manitou)			
	Tubers per plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)	Tubers per plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)	Tubers per plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)
Low PCN infestation												
Oxamyl	6.7	50.4	21.03	0	12.2	77.0	33.18	0	8.5	86.9	35.71	0
Fluopyram	5.9	44.6	17.88	14.98	12.1	71.1	30.51	8.05	8.5	81.5	34.56	3.22
Azadirachtin	6.3	38.7	15.71	25.30	12.6	57.6	24.78	25.32	8.7	70.2	29.54	17.28
P. lilacinus	6.1	36.7	14.40	31.53	12.4	55.4	23.05	30.53	8.1	64.4	27.06	24.22
Control	6.5	24.8	9.89		12.4	24.4	8.04	75.77	8.6	36.61	13.84	61.24
Moderate PCN infestation												
Oxamyl	5.6	49.3	20.46	0	12.1	73.6	31.96	0	8.3	86.0	35.58	0
Fluopyram	6.1	42.1	17.53	14.32	12.4	67.1	29.45	7.8	8.4	76.4	32.51	8.63
Azadirachtin	6.1	36.7	15.11	26.15	12.7	58.1	25.33	20.74	8.6	64.4	27.30	23.27
P. lilacinus	6.6	30.1	12.27	40.03	12.3	54.2	22.79	28.69	8.2	60.3	25.82	27.43
Control	5.9	16.1	5.84	71.46	12.4	19.4	6.43	79.88	8.2	32.9	12.91	63.72
High PCN infestation												
Oxamyl	5.9	47.7	19.89	0	12.2	71.7	31.19	0	8.4	82.0	34.99	0
Fluopyram	6.5	40.5	17.24	13.32	12.3	62.2	26.52	14.97	8.2	57.6	23.82	31.92
Azadirachtin	6.6	25.7	10.25	48.47	12.3	51.8	21.24	31.90	8.4	59.4	24.20	30.84
P. lilacinus	6.0	22.7	9.15	54.0	11.9	47.8	19.84	36.39	8.8	55.4	23.30	3.41
Control	6.6	13.6	5.50	72.35	12.8	14.0	5.72	81.66	8.5	27.9	10.41	70.25

For number of tubers per plant: CV (%) =4.6, LSD $_{Variety}$=0.22*

For tuber weight CV (%) =19.32%, LSD $_{PCN\ control\ product}$=5.26*; LSD $_{variety}$=4.07*; LSD $_{site}$=4.07*, LSD $_{PCN\ control\ product\ x\ variety}$=9.10*; LSD $_{PCN\ control\ product\ x\ site}$=15.76^{NS}, LSD $_{variety\ x\ site}$=9.10^{NS}; PCN control product x variety x site =7.05^{NS}

For marketable yield CV (%) =17.7%, LSD $_{PCN\ control\ product}$=0.08*; LSD $_{variety}$=0.06*; LSD $_{site}$=0.06*; LSD $_{PCN\ control\ product\ x\ variety}$=0.18*; LSD $_{PCN\ control\ product\ x\ site}$=0.18*; LSD variety x site=0.14^{NS}; PCN control product x variety x site =0.31*

NS-Not significant; * significant at p=0.05

Table 5.5. Effect of potato cyst nematode (PCN) control products, infestation levels and variety on number of tubers per plant, mean tuber weight and marketable yields during the short rains season 2021

Level of PCN infestation and treatments	Susceptible variety				Susceptible variety				Resistant variety			
	Tubers per Plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)	Tubers per plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)	Tubers per plant	Mean tuber weight (g)	Marketable yields (t ha^{-1})	Relative Marketable yield loss (%)
Low PCN infestation												
Oxamyl	6.4	46.1	18.80	0	12.0	73.0	28.27	0	8.6	77.9	30.76	0
Fluopyram	5.9	41.6	16.85	10.37	12.4	64.4	27.14	4.00	8.1	68.0	28.18	8.39
Azadirachtin	5.9	35.4	13.30	29.26	12.4	45.8	18.57	34.31	8.5	67.2	22.05	28.32
P. lilacinus	6.2	30.8	11.52	38.99	12.3	48.9	17.35	38.63	8.3	55.4	20.50	3.36
Control	5.7	27.0	10.10	46.28	11.9	28.9	10.89	61.48	8.4	28.4	9.06	70.55
Moderate PCN infestation												
Oxamyl	6.2	41.2	16.49	0	12.2	67.9	28.69	0	8.1	75.8	32.04	0
Fluopyram	6.3	38.5	14.98	9.16	12.3	60.3	25.47	11.22	8.6	67.3	28.16	12.11
Azadirachtin	6.0	26.1	10.69	35.17	12.4	47.9	20.24	29.45	8.5	58.7	22.02	31.27
P. lilacinus	6.2	22.9	9.14	44.57	11.6	39.2	16.17	43.64	8.6	45.0	19.15	40.23
Control	6.1	19.7	7.88	52.21	12.2	22.4	9.06	68.42	8.1	25.3	7.21	77.50
High PCN infestation												
Oxamyl	5.9	40.3	15.05	0	12.4	66.6	25.49	0	8.3	70.9	25.34	0
Fluopyram	6.3	38.0	14.14	6.05	12.6	55.0	21.76	14.63	8.3	63.9	25.22	0.47
Azadirachtin	5.9	21.1	8.28	44.98	12.4	31.6	9.37	63.24	8.5	55.8	20.57	18.82
P. lilacinus	6.2	15.0	6.07	59.67	12.1	26.8	8.96	64.85	8.4	41.2	18.84	25.65
Control	6.5	13.9	5.54	63.18	12.5	17.9	2.82	88.93	8.2	19.9	7.40	70.80

For number of tubers per plant: CV (%) =4.1, LSD $_{Variety}$=0.15*

For tuber weight CV (%) =36.9%, LSD $_{PCN\ control\ product}$=5.95*; LSD $_{variety}$=4.62*; LSD $_{site}$=4.62*, LSD $_{PCN\ control\ product\ x\ variety}$=10.32*; LSD $_{PCN\ control\ product\ x\ site}$=10.32^{NS}, LSD $_{variety\ x\ site}$=7.99^{NS}; PCN control product x variety x site =17.88^{NS}

For marketable yield CV (%) =40.4%, LSD $_{PCN\ control\ product}$=3.70*; LSD $_{variety}$=2.87*; LSD $_{site}$=2.87*; LSD $_{PCN\ control\ product\ x\ variety}$=6.41*; LSD $_{PCN\ control\ product\ x\ site}$=6.41*; LSD variety x site=4.97^{NS}; PCN control product x variety x site =11.11*

NS-Not significant; * significant at p=0.05

In general, the control plots had the lowest total mean tuber weights regardless of variety or level of PCN infestation in both seasons. Application of Oxamyl for the control of PCN led to the highest mean tuber weights compared to the other PCN control products during both seasons regardless of the variety or level of PCN infestation. Variety Desiree had the lowest mean tuber weights for each of the PCN control treatments applied compared to the other two varieties. For example, during the long rains 2021 plots treated with Oxamyl in a high PCN infested site had mean tuber weights of 47.7 g/tuber (Desiree), 71.7 g/tuber (Shangi) and 82.0 g/tuber (Manitou) A similar observation was made during the short rains season where the mean tuber weights in plots treated with oxamyl in a high PCN infested area were 40.3 g/tuber (Desiree), 66.6 g/tuber (Shangi) and 70.9 g/tuber (Manitou).

5.4.3.3 Marketable yield

During both seasons, the effects of PCN control product, variety, and site on marketable yield were significant. The interaction between PCN control product and variety on marketable yield was only significant during the long season but not during the short rains season. The effects of the interactions of PCN control product x site, variety x site and PCN control product x variety x site on marketable yield were not significant during both seasons.

Marketable yields varied from 5.5 (variety Desiree planted in a heavily PCN infested site but without application of any nematicides) to 35.7 t ha^{-1} (variety Manitou planted in a low PCN infested site but treated with oxamyl) during the long rains season. During the short rains season they varied from 2.8 (variety Shangi planted in a heavily PCN infested site and without application of nematicides) to 32.0 t ha^{-1} (Variety Manitou planted in a moderately infested PCN site and treated with oxamyl) (Table 5.4 and 5.5).

The control plots had the lowest marketable yields regardless of variety or level of PCN infestation in both seasons. Control of PCN by application of oxamyl resulted in the highest marketable yields compared to the other PCN control products during both seasons regardless of the variety or level of PCN infestation.

In general, the lowest marketable yields for each of the PCN control treatments applied were recorded in variety Desiree compared to the other two varieties. For example, during the long rains 2021 plots treated with oxamyl in a high PCN infested site had marketable yields of 19.9 t ha^{-1} (Desiree), 35.0 t ha^{-1} (Manitou) and 31.2 t ha^{-1} (Shangi). A similar observation was made during the short rains season. In plots treat with oxamyl in a high PCN infested area the marketable yields were 15.1 t ha^{-1} (Desiree), 25.2 t ha^{-1} (Manitou) and 25.5 t ha^{-1} (Shangi). However, during the short rains season, variety Shangi grown in a heavy PCN infested site but without application of any PCN control product had the lowest marketable yields (2.9 t ha^{-1}) compared to the other two varieties.

5.4.3.4 Tuber yield

The effects of the interactions of PCN control product x variety, PCN control product x site, variety site and PCN control product x variety x site were not significantly different. Total yields varied from 6.06 to 38.62 t ha^{-1} and from 6.16 to 34.60 t ha^{-1} during the long and short rain 2021 seasons, respectively (Tables 5.4 and 5.5). In general, the uncontrolled plots had the lowest total tuber yields regardless of variety or level of PCN infestation in both seasons. Application of oxamyl for the control of PCN led to the highest total tuber yield compared to the other PCN control products during both seasons regardless of the variety or level of PCN infestation (Tables 5.4 and 5.5).

In general, variety Desiree had the lowest total tuber yields for each of the PCN control treatments applied compared to the other two varieties. Among the PCN control products oxamyl had the highest yield increase at all levels of infestations and among the three varieties**.** For example, during the long rains 2021 plots treated with oxamyl in a high PCN infested site had yields of 21.21 t ha^{-1} (Desiree), 31.85 t ha^{-1} (Shangi) and 36.43 t ha^{-1} (Manitou). A similar observation was made during the short rains season where plots treated with oxamyl in a high PCN infested area had yields of 17.81 t ha^{-1} (Desiree), 29.61 t ha^{-1} (Shangi) and 31.50 t ha^{-1} (Manitou) (Table 5.4 and 5.5).

5.4.3.5 Relative marketable yields

Relative marketable yield was significantly influenced by potato cyst nematode control product, variety, and site during the long and short rains season, 2021. The effects of the interactions of PCN control product x variety, PCN control product x site, variety site and PCN control product x variety x site were not significant. In general, the uncontrolled plots had the lowest percent marketable yields regardless of variety or level of PCN infestation in both seasons.

All PCN control products caused a significant low relative marketable yield loss compared to the untreated plots. For example, during the long rains season, the treated plots of Shangi with fluopyram grown in low PCN infested site had a relative marketable yield of loss of 4%, while relative marketable yield loss of the untreated plots was 61.48%. A similar observation was made during the short rains season where potato cultivar Desiree grown in treated plots with fluopyram in heavily PCN infested soil had a relative marketable yield loss of 6.05 while the untreated plot had 63.18%.

5.4.3.6 Relative yield loss

Overall, tuber yield losses were significantly reduced on potato varieties treated with PCN control products over the untreated control plots. The percent relative yield loss range as a result of application of the PCN control products were 47.4-93.7 (fluopyram), 40.2 – 80.8 (Azadirachtin), 71.9 - 94.5 (*P. lilacinus*) and 19.5-39.6 (control) in the two seasons compared to the best performing treatment oxamyl in each specific variety (Tables 5.6 and 5.7).

Among the three varieties, Manitou had the lowest relative yield loss in both seasons. During the long rains 2021, the range in percent relative yield loss was 6.3 % (Variety Manitou treated with fluopyram and grown in a site with low PCN infestation) to 80.5% (Variety Shangi with no PCN control products grown in a site with moderate PCN infestation levels). During the short rains 2021 season the % relative yield loss varied from 5.5 % (variety Desiree grown in a site with high PCN infestation and treated with fluopyram) to 77.0% (Variety Shangi with no PCN control products grown in a site with moderate PCN infestation levels). The % relative yield loss was dependent on the PCN control product applied, variety and the levels of PCN infestation. PCN control products that gave high yields resulted in relatively low yield loses and vice versa.

Table 5.6. Yield (t ha^{-1}) and relative yield loss (%) of different potato cultivars grown in fields infested with varying population densities of potato cyst nematodes (PCN), during the long rains of 2021

Level of PCN infestation And treatments	Susceptible variety (Desiree)		Susceptible variety (Shangi)		Partially resistant variety (Manitou)	
	Total yield (t ha^{-1})	Relative yield loss (%)	Total yield (t ha^{-1})	Relative yield loss (%)	Total yield (t ha^{-1})	Relative yield loss (%)
Low PCN infestation						
Oxamyl	22.4	-	34.2	-	38.62	-
Fluopyram	19.8	11.6	31.6	7.7	36.2	6.3
Azadirachtin	17.2	23.2	25.6	25.2	31.2	19.2
P. lilacinus	16.3	27.2	24.61	28.1	28.6	25.9
Control	11.3	49.6	10.83	68.4	16.27	57.9
Moderate PCN infestation						
Oxamyl	21.9	-	32.69	-	38.2	-
Fluopyram	18.71	14.6	29.8	8.8	33.96	11.1
Azadirachtin	16.29	25.6	25.81	21.0	28.6	25.1
P. lilacinus	13.4	38.8	24.1	26.3	26.81	29.8
Control	7.15	67.4	8.62	73.6	14.61	61.8
High PCN infestation						
Oxamyl	21.21	-	31.85	-	36.43	-
Fluopyram	18.01	15.1	27.62	13.3	25.6	29.7
Azadirachtin	11.43	46.1	23.01	27.8	26.41	27.5
P. lilacinus	10.07	52.5	21.24	33.3	24.6	32.5
Control	6.06	71.4	6.22	80.5	12.4	66.0

Data are means of three replications

For total yield: CV (%) =19.3, $LSD_{PCN\ control\ product}$ =2.3*; $LSD_{variety}$=1.8*, LSD_{site}=1.8*, $LSD_{PCN\ control\ product\ x\ variety}$=4.0*; $LSD_{PCN\ control\ product\ x\ site}$=$4.0^{NS}$, $LSD_{variety\ x\ site}$=3.1^{NS}; PCN control product x variety x site =7.0^{NS}

NS-Not significant; * significant at ($p \leq 0.05$)

Table 5.7. Yield (t ha^{-1}) and relative yield loss (%) of different potato cultivars grown in fields infested with varying population densities of potato cyst nematodes (PCN), during the short rains of 2021

Level of PCN infestation And treatments	Susceptible variety (Desiree)		Susceptible variety (Shangi)		Partially resistant variety (Manitou)	
	Total yield (t ha^{-1})	Relative yield loss (%[1])	Total yield (t ha^{-1})	Relative yield loss (%[1])	Total yield (t ha^{-1})	Relative yield loss (%[1])
Low PCN infestation						
Oxamyl	20.50	-	32.45	-	34.60	-
Fluopyram	18.50	9.8	28.60	11.9	30.20	12.7
Azadirachtin	15.70	23.4	20.36	37.3	29.88	15.6
P. lilacinus	13.68	33.4	21.74	33.0	24.68	29.7
Control	11.98	41.6	12.85	60.4	12.60	63.6
Moderate PCN infestation						
Oxamyl	18.29	-	30.17	-	33.70	-
Fluopyram	17.20	16.0	26.80	11.2	29.90	11.3
Azadirachtin	11.60	27.6	21.29	29.4	26.09	22.6
P. lilacinus	10.19	44.8	17.42	42.3	20.00	40.7
Control	8.76	52.1	9.97	77.0	11.25	66.6
High PCN infestation						
Oxamyl	17.89	0.0	29.61	0.0	31.50	0.0
Fluopyram	16.90	5.5	24.44	17.5	28.40	9.8
Azadirachtin	9.36	47.7	14.03	52.6	24.80	21.3
P. lilacinus	6.67	62.7	11.90	59.8	18.01	42.8
Control	6.19	65.4	7.90	73.3	8.83	72.0

Data are means of three replications, For total yield: CV (%) =26.9, $LSD_{PCN\ control\ product}$ =3.8*; $LSD_{variety}$=3.0*, LSD_{site}=3.0*, $LSD_{PCN\ control\ product\ x\ variety}$=6.7*; $LSD_{PCN\ control\ product\ x\ site}$=$6.7^{NS}$, $LSD_{variety\ x\ site}$=5.2^{NS}; PCN control product x variety x site =11.7^{NS}NS-Not significant; * significant at $(p \leq 0.05)$

5.4.4. Correlation between cyst populations and yield

The relationship between the final number of cysts, the reproduction index and total tuber yield is presented in Table 5.8. A positive correlation (r= 0.4640, r= 0.4802) ($p \leq 0.05$) between final cysts and reproduction index during long and short rains respectively was observed. Additionally, final cysts were significantly ($p \leq 0.05$) and negatively (r=-0.4987, r=-0.5085) correlated with total tuber yield during long and short rains season, respectively. The reproductive index was significantly ($p \leq 0.05$) and negatively (r=-0.662, r=- 0.3675) correlated with total tuber yield during long and short rain season (Table 5.8).

Table 5.8. Correlation coefficients describing the relationship between final cysts number, reproduction index and tuber yields during the long and short rains seasons 2021

Variable	Long rains 2021			Short rains 2021		
	Final cysts count	Reproduction Index	Tuber yield	Final cysts count	Reproduction Index	Tuber yield
Final cysts count	-			-		
Reproduction Index	0.4640*	-		0.4802*	-	
Tuber yield	-0.4987*	-0.6621*	-	-0.5085*	-0.3675*	-

*=significant at ($p \leq 0.05$)

5.5 Discussion

This study has demonstrated that PCN infestations at different population densities tested caused significant yield losses of up to 80.5% when not controlled. Use of nematicides and host resistance was observed to be effective in lowering the number of final cysts and PCN reproduction resulting to high yield. The low PCN multiplication rate resulted in low yield loss among the PCN control products. This could be partly due to reduction of the initial population of PCN densities which results in to low root invasion. These results are in line with other studies (Hedfi, 2017; Minnis *et al.,* 2004; Djebroune *et al.,* 2020) which reported a reduction in PCN reproduction and increase in yields when nematicides and resistant potato varieties were used to manage PCN.

Among the PCN control products used in this study, oxamyl was superior in final cysts reduction over fluopyram, Azadirachtin and *P. lilacinus* in reducing nematode infestation, leading to lower yield loss. This effect could be partly due to reduction of initial population densities of PCN by oxamyl leading to low root invasion, suppressive activities on multiple nematode life stages of juveniles by restricting hatching and activities in locating feeding sites in the roots and limited movement of juveniles causing mortality thus resulting to less juveniles drawing nutrients from plants (Hedfi, 2017). These results corresponds with previous research (Hedfi, 2017; Minnis *et al.*, 2004) which found out that using oxamyl to manage PCN reduced its reproduction while increasing yields. It should however be noted that oxamyl has recently been banned in several countries due to its toxicity (https://www.farminguk.com/news/ahdb-applies-for-emergency-approval-of-vydate-after-ban_57311.html) in Kenya it was withdrawn from the market in 2022 and may not be available for Kenyan farmers in the near future. Hence the need to focus more on safer approaches to manage the pest. Potato farmers have the option to choose from biological and safe synthetic products like floupyram to manage the pest. They must however, carefully weigh the economic and environmental benefits and drawbacks of each option. An ideal nematode management program must be competitively priced in order to maintain high returns on investments, but must also increase yield and thus revenue through either mitigating nematode damage or stimulating plant production or both.

A consistently high decline in multiplication rate of PCN was observed in variety Manitou at all levels of initial population densities and seasons. Use of the resistant varieties, results in second stage juveniles unable to form feeding sites, which leads to their starvation and mortality. This could be as a result of H1 gene which is reported to be present and confers partial resistance, allowing the nematodes to multiply in slightly lower rates (Mburu *et al.*, 2020). However, use of host resistance alone has limited scope for control of PCN due to selection for virulence in PCN population. Turner and Stone, (2019) showed distinct selection of virulence strains of *G. pallida* after eleven generations of planting partially resistant varieties to *G. pallida* in one field. Therefore, PCN virulence strain would increase if resistance varieties were grown, hence a combination of both PCN control products and host resistance would be sustainable in reducing PCN reproduction and reduction of yield loss. Of particular importance is variety Manitou which when treated with oxamyl resulted in 100 % reduction in cysts numbers leading to lower yield losses compared with other varieties.

Furthermore, the study found that cultivars Shangi and Desiree consistently had high PCN population densities and reproduction indexes across the three levels of infestations. Mburu *et al.*, (2020) discovered that both varieties lacked the H1 gene, which confers resistance to *G. rostochiensis* making the two varieties susceptible to *G. rostochiensis*. These findings agreed with those of Mezerket *et al.*, (2018) who reported that Desiree as a susceptible host of *Globodera* spp. and gave low yields compared to other varieties. Furthermore, cultivar Shangi which is the most commonly grown variety by smallholder farmers is susceptible to *G. rostochiensis*. Due to scarcity of land, the smallholder farmers subject available land to intensive cultivation of variety Shangi with hardly any meaningful crop rotation being practiced. The problem of PCN build up and spread is compounded by the fact that, smallholder farmers normally use uncertified potato planting materials.

The levels of PCN reproduction were found to be inversely related to the initial infestation level (Pi) of trial sites in the current study. This relationship between intial population and reproductive index was observed in both susceptibele and resistant cultivars. This resulted in higher yield losses in low PCN infested site. This phenomena may be attributed to intraspecific competition for nutrients and space in roots. Whereby low inoculum in the field lead to less competition for finding host, nutrients and feeding sites, resulting to a high PCN invasion in the roots. This observation was made on all the varieties when grown in low PCN infestation without nematicide where a high reproductive index of above 1.62 was recorded. According to Trudgill (1986), PCN sex is determined nutritionally where relatively more females are able to mature as females in low PCN infested farms. Only at moderately and heavily infested sites was the variety Manitou able to reduce PCN without nematicides. A similar increase in cysts multiplication and yield reduction in a low PCN infested field was reported by Maneva and Trifonov (2015). This implies that pre-plant PCN population (Pi) have an impact on yield loss of potato crop.

The findings of this study show a negative correlation between final PCN population densities (Pf) and yield, reproduction index and yield. This could be due to the high number of PCN infestation resulting to rapturing of more cells in the root cortex and vascular system in the roots which result to ineffective nutrient and water uptake ultimately forming small and fewer tubers. Studies by Djebroune *et al.* (2020) had a similar observation, when Kondor - a resistant variety- was grown

in a PCN infested field, and the final cysts were significantly reduced resulting in a significant yield gain.

PCN infection significantly reduced marketable and relative marketable yields. For example, relative marketable yield loss ranged from 3.22 to 81.66 % and 4.00 to 88.93% during long rains and short rains, respectively. These results are in line with those of Grabau *et al*. (2019) who reported that marketable yields increased between 49 to 66%, 33 to 55% and 41 to 61% in nematicide treated plots relative to the untreated control in three different years. Lack of accuracy of methods used to estimate crop losses brought on by pests and diseases in general, and plant-parasitic nematodes in particular, are frequently criticized (Minnis *et al.,* 2004). The debate over the role of ‘‘other pests and pathogens’’ present in the field besides the target pest is occasionally justified. However, in the current study, the impact of other pests and diseases, if any, was uniform across the treated and untreated plots and thus, had no impact on yield losses reported in this study. Conducting yield loss trials involving only the target pest in ‘‘closed environments’’ is considered unrealistic because nematode effects are liable to being influenced by prevailing biotic and abiotic stresses under field conditions (Kumar *et al.,* 2020).

Significant export losses may result from *G. rostochiensis* becoming endemic in Kenya, particularly if pest freedom status is lost, resulting in future exports prohibition. Many potential destinations for future exported potato from Kenya are largely PCN free and may restrict imports resulting in potentially loss of incomes. In addition to losing area-freedom status, any increased chemical use could restrict future market access, particularly in new and emerging markets. Export of any crops that are potential hosts of *G. rostochiensis* (tomato and eggplant or aubergine), as well as crops grown in soils potentially containing *G. rostochiensis* may also be affected. Previous studies have shown that majority of farmers in Kenya do not control potato cyst nematodes (Chapter 4; Mburu *et al*., 2020). This means that they potentially incur heavy losses as demonstrated by the yield losses.

The interaction between the PCN control products and the resistant variety was evident by reducing PCN multiplication and yield losses significantly. This approach of use of PCN control products and tresistance will improve PCN management. Although, the biopesticides used in this study were not as effective as synthetic nematicides, they outperformed the control, indicating that

there is promise in the use of biopesticides in management of potato cyst nematodes. This is the first study to measure the impact of PCNs on yield losses of rain-fed potato in the country and is among the few studies conducted on potato cyst nematodes under natural infestations in Kenya.

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CHAPTER 6: EFFICACY AND ACTIVE INGREDIENTS OF SELECTED PLANT EXTRACTS ON POTATO CYST NEMATODES (*GLOBODERA ROSTOCHIENSIS*) UNDER *IN-VITRO* AND *IN-VIVO* CONDITIONS

6.1 Abstract

The potential of plant extracts (PE) to inhibit potato cyst nematodes (PCN) (*Globodera spp*.) is yet to be fully exploited as an alternative to synthetic nematicides. In search for alternatives to chemical control of PCN, a set of two experiments were conducted under *in vitro*, screenhouse and field conditions in 2021. In the first experiment, extracts from Mexican marigold *Tagetes minuta),* Mexican sunflower (*Tithonia diversifolia),* garlic (*Allium sativum),* blue gum (*Eucalyptus grandis* (leaves and bark), spring onion (*Allium fistulosum*) leaves, onion bulb (*Allium cepa*), ginger *(Zingiber officinale*), green tea leaves (*Camellia sinensis*) and Sodom apple (*Solanum incanum*) were tested under *invitro* conditions. Neem (*Azadirachta indica*) extract was the standard whereas potato root diffusate was the negative control. The solvents used in extraction of phytochemicals were methanol, ethyl acetate, hexane and water. Eggs and J2s of PCN were exposed to the PEs for 24, 48 and 72 hrs. Treatments were arranged in a completely randomized design (CRD) with three replications. In the second experiment; the effects of hexane extracts from ginger, garlic and Mexican sunflower at 25, 50, 100 and 150 mg/ml were evaluated against PCN under screenhouse and field conditions. Oxamyl (Vydate) and commercial neem extract (Achook) treatments were included as positive controls while the negative control was distilled water. In the screenhouse experiment, the experiments were arranged in a completely randomized design (CRD) with Shangi as the test variety. The field experiments were carried out in soils with natural infestations of PCN using a randomized complete block design (RCBD). Only 3 extracts (ginger, Mexican sunflower, and garlic) showed a high and consistent number of non-viable egg counts in the *invitro* experiments. Mexican sunflower resulted in a significantly higher loss of egg viability having 93 and 89.2 % non-viable eggs/cyst in experiment 1 and 2, respectively compared to the other PEs. Garlic, Mexican sunflower and ginger after 72 hrs of treatment exposure had significantly ($P<0.05$) high juvenile mortalities of 64.5 %, 64.9 % and 70.2 %. In the screenhouse and field experiments, ginger extracts at 150 mg/ml had the lowest PCN reproduction indexes of 0.10 and 0.17 during the short and long rains, respectively. In both seasons, the total PCN population was significantly ($p \leq 0.05$) reduced in all the treatments with the reproduction index ranging from 0.50-0.70 while potato yield was significantly increased compared to the control. Ginger extract, applied at 100

mg/ml, increased yield by 112.2 and 80.6 % during the short and long rains, respectively. Hexane extracts of ginger had the highest percentage of alkaloids, saponins, flavonoids, phenols, terpenoids, glycosides and tannins among the plant extracts evaluated. This study has demonstrated that PEs from Mexican sunflower, ginger and garlic are effective in inducing loss of egg viability and causing mortality of PCN J2s. They therefore, have potential to be incorporated in integrated management of potato cyst nematodes in potato production.

6.2 Introduction

Potato cyst nematodes (PCN) (*Globodera rostochiensis*) can be managed through various ways which include crop rotation, application nematicides, organic amendments, biological and botanical extracts and use of resistant varieties (Khan and Kim, 2007). Farmers have however relied on synthetic pesticides to manage pests worldwide (Pylypenko, 2018). There are however, concerns about their environmental effects, resistance to pests, high residual effects in crops, health hazards to farmers and the associated costs for smallholder farmers which limit their use (Chitambo, 2019). Consequently, alternative approaches of nematode control such as use of antagonistic plants are increasingly being explored and implemented for management of nematodes to reduce the risks associated with synthetic chemicals (Humaira *et al.,* 2020). They produce secondary metabolites (phytochemicals) that have potential use as botanical pesticides to control. These phytochemicals suppress pathogens through allelopathic interactions to inhibit reproduction, growth and biological activity (Bhattacharyya, 2017). Botanical pesticides are non-persistent since they are converted through oxidation, light and micro-organisms into less toxic products (Taniwiryono *et al.,* 2009).

Plants extracts belonging to about 57 families including *Meliaceae*, *Asteraceae*, *Myrtaceae*, *Amaryllidaceae*, *Theaceae*, *Zingiberaceae*, and *Alliaceae* are reported to possess either nematicidal or insecticidal activity (Boulogne *et al.,* 2012; Okwute, 2012). The majority of plants belonging to these families contain phytochemicals such as alkaloids, fatty acids, phenols, polyacetylenes, sesquiterpenes, glucosinolates thienyls, isothiocyanates and diterpenes *(*Ansari *et al.,* 2020; Khan *et al.,* 2019). These metabolites confer fungicidal, nematicidal, insecticidal, acaricidal and bactericidal properties to these extracts. Previous research has shown that plant extracts from Mexican marigold (Bhattacharyya, 2017), Ginger (Amer-Zareen *et al.,* 2003),

Eucalyptus spp and onion bulb (Cetintas and Qadir, 2014), Spring onions (Salifu *et al.*, 2019) and Sodom apple (Waweru *et al.*, 2017) have nematicidal effects against root knot nematodes. Sultana and Khan, (2018) found that plant extracts from tea were effective in controlling the plant parasitic nematodes *Helicotylenchus indicus*, *Xiphinema americanum* and *Xiphinema index*, whereas crude garlic extracts were effective against *Globodera pallida* in potato fields (Danquah *et al.*, 2011). Solvents are important in the efficacy of bioactive compounds found in plants extracts. Furthermore, phytochemicals solubility varies with phytochemical constituent's polarity in a plant and this can only be extracted with a suitable solvent (Gurnani *et al.*, 2016). Despite the potential of plant extracts for nematode control, there is paucity of information on their effectiveness in managing PCN in Kenyan agro-ecological conditions characterized by continuous potato cultivation. The aim of the study was to investigate the nematicidal potential of plant extracts from ten locally available plant species obtained using different solvents for their efficacy of management of eggs and second stage juveniles (J2s) of the potato cyst nematodes under *in-vitro* and *in-vivo* conditions.

6.3 Material and methods

6.3.1 Collection of plant materials

The plants materials categorised as extract 1, 2, 3, 4, 5, 7, 8, 9 and 10 (Table 6.1) were used for the study. The plants were collected from Kenya Agricultural and Livestock Research Organization (KALRO) Tigoni, Limuru market (1°6'0"S, 36°39'0"E), and farms around Juja (1° 11' 0" S, 37° 7' 0" E) in Kenya. The plants were selected on the basis that they possess nematicidal activity against *M. incognita* (Bhattacharyya, 2017; Cetintas and Qadir, 2014; Waweru *et al.*, 2017). Neem (*Azadirachta indica*, Meliaceae) extract was used as the standard whereas potato root diffusate was included as the negative control.

6.3.2 Preparation of plant extracts

Plant extracts from three different plants were prepared using a modified technique described by Chang *et al.*, (1977). Plant species were decontaminated by separately washing them thoroughly using water and then spread on a polythene sheet to air dry for 10 days on a bench in a shaded screenhouse. Approximately 200 g of air-dried material from a sample of each plant species were

Table 6.1. Plant extracts and checks evaluated for nematicidal activity against eggs and second stage juveniles (J2s) of the potato cyst nematodes

Extract id.	Test plant extracts /product	Scientific name	Family	Part extracted	Previous work
1	Mexican marigold	*Tagetes minuta*	Asteraceae	Leaves	(Bhattacharyya, 2017)
2	Mexican sunflower	*Tithonia diversifolia*	Asteraceae	leaves	(Odeyemi and Adewale, 2011)
3	Garlic	*Allium sativum*	Amaryllidaceae	Bulb	(Danquah, 2011)
4	Eucalyptus leaves	*Eucalyptus grandis*	Myrtaceae	Leaves	(Cetintas and Qadir, 2014)
5	Eucalyptus bark	*Eucalyptus grandis*	Myrtaceae	Bark	(Cetintas and Qadir, 2014)
6	Spring onion	*Allium fistulosum*	Amaryllidaceae	Leaves	(Salifu *et al.,* 2019)
7	Sodom apple	*Solanum incanum*	Solanaceae	Fruit	(Waweru *et al.,* 2017)
8	Green tea leaves	*Camellia sinensis*	Theaceae	Leaves	(Sultana and Khan, 2018)
9	Ginger	*Zingiber officinale*	Zingiberaceae	Corm	(Amer-Zareen *et al.,* 2003)
10	Onion bulb	*Allium cepa*	Amaryllidaceae	Bulb	(Cetintas and Qadir, 2014)
11	Neem (Positive control)	Commercial neem extract	Meliaceae		(Trifonova and Atanasov, 2011)
12	Potato root diffusate (Negative Control)		*Solanum Tuberosum*	Roots	(EPPO, 2017)

then macerated into a fine powder using a Phillips kitchen blender. To prepare the extracts, 60 g powder of each sample was soaked in 200 ml of 4 solvents (water, methanol, ethyl acetate and hexane) separately n a 500 ml beaker and incubated for 12 hours at room temperature (approximately 20^{o}C). The concentration of solvents was 100 %. Following that, the extracts were then filtered through Whatman filter paper and the filtrates subjected to evaporation using a soxhlet extractor at 70 ^{o}C for 30 min. The collected solution was placed in sand bath at 70 ^{o}C for 72 hours and concentrated extracts were obtained. This process was repeated until the required yield of pellets for each of the plant extracts was obtained. The pellets were then stored in an airtight container in a refrigerator at 10 ^{o}C. Pellets (approximately 5 g) were then dissolved in 200 ml of dH_2O resulting in a concentration of 25 mg/ml before use (Babaali *et al.*, 2017). Thereafter, three more concentrations were prepared 50 mg/l, 75 mg/l and 100 mg/l. The remaining pellet was used for phytochemical analysis.

6.3.3 Preparation of potato cyst nematode (PCN) inoculum

Potato cyst nematodes were extracted from heavily infested soil in Nyandarua county fields. The cysts were extracted using the Fenwick can floatation method (Fenwick, 1940) at KALRO Tigoni. Cysts were then picked from the extracted samples under a binocular microscope at 40X magnification. For the screenhouse experiment 30 cysts for each pot were counted and preserved in the vial until when needed.

To prepare second stage juveniles (J2s) for *in-vitro* assay, potato root diffusate (PRD) was used as a hatching medium (EPPO Bull, 2017). The PRD was obtained by pouring 1000 ml of tap water in a 3-week-old potted potato plant growing in soil. The PRD obtained was sieved using muslin cloth, 5 ml of which was put into a 10 well hatching plate (EPPO, 2017). Five hundred cysts were then added in the 5 ml PRD ensuring all cysts were completely submerged. The plate was then kept in the dark for seven days and examined for juveniles that had emerged. The juvenile suspension obtained in the hatching vessel was then put in a 200 ml beaker and homogenized using an MRC magnetic stirrer for three minutes. From the juvenile suspension, a 0.5 ml PRD containing 100 infective juveniles was prepared for each treatment including the control in a 24 well plate. Counting of the juveniles was done under a Leica stereoscopic microscope at 40x magnification.

6.3.4 *In vitro* assays

The *in-vitro* assays were conducted at ICIPE Nematology Laboratory (25 ± 5°C). Both experiments were arranged in a Completely Randomized Design (CRD) with three replications per treatment and incubated for 24, 48 and 72 hrs. The experiments were repeated once.

Experiment 1. Determining PCN egg hatchability and viability when exposed to plant extracts at different time intervals

On average, three cysts were recovered from 100 g of soil. Therefore, for the egg hatchability and viability test, three cysts of PCN were picked from already extracted samples and transferred into each of the 96 well plates. Aliquots of 250 µl of each of the ten plant extracts from each solvent (methanol, ethyl acetate, hexane and water), negative control (PRD) and the positive control - Achook (neem based extract) from Organix Limited was prepared following manufacturer's recommendations. They were dispensed into each well ensuring all the three cysts were well immersed. Each treatment had three cysts and was replicated three times. To avoid evaporation, all 96 well plates were covered with aluminum foil and kept in the dark, inside laboratory cabinets at 25 ± 5°C. After the designated time (24, 48 and 72 hrs) of exposure had elapsed, one cyst was picked from each experimental unit in each treatment, after which the cysts were incubated in 0.1 % Nile blue stain for 48 hrs (Faggian *et al.*, 2012; Kroese *et al.*, 2011).

Experiment 2. Determining the mortality rate second stage PCN juveniles upon exposure to different plant extracts at varying durations

For the juvenile mortality test, 0.5 ml PRD having 100 freshly hatched J2s (Asif *et al.*, 2015; Fatemy, 2018) of PCN were added in each of the 24 well plates containing 1.5 ml of the 10 plant extracts with the negative control (PRD) and the positive control Achook (neem based extract) from Organix Limited also included. The positive control was prepared according to manufacturer's recommendations. Each treatment had three experimental units and was replicated three times. Wrapped in aluminum foil, the well plates were incubated at 25°C for 24, 48, and 72 hours hrs. The experiment was done twice.

Following incubation, the aliquots in each well were homogenized and 2 ml was drawn and put on a counting dish to determine the number of both live and dead J2s counted under a Leica stereoscopic microscope after 24, 48 and 72 hrs. J2s showing any mobility (active) were considered living and those showing no movement were considered dead, they were probed with a surgical needle to ascertain their status (Cayrol *et al.,* 1989).

6.3.5 *In-vivo* experiments

Screenhouse experiment

The study was carried out at Kenya Agricultural and Livestock Research Organisation (KALRO) –Tigoni (1°08'S and 36°40'E), at an altitude of 2,100 m, under screenhouse and field conditions during the long and short rainy seasons of 2021.

Well sprouted potato tubers of cultivar Shangi were planted in 1kg pots containing a steam sterilized mixture of soil in a ratio of 3:2:2:1 for manure, soil, sand and ballast, respectively in the screenhouse maintained at 25 ± 5 °C. At planting, 10 g of fertilizer was added in each pot. Two weeks after planting, 30 cysts of PCN having about 100 eggs/cyst were then inoculated in each of the pots including the control. The treatments consisted of hexane extracts from ginger, garlic and Mexican sunflower at concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml, oxamyl (8 l/ha) and Azadirachtin (6 l/ha) and distilled water (negative control) and were added as a soil drench seven days after inoculation of PCN. Oxamyl (8 l/ha) and Azadirachtin (6 l/ha) were used as standard controls while distilled water was the negative control. Approximately 50 ml of each treatment was applied in the respective pot. The trial was arranged in a completely randomized design (CRD) and the treatments were replicated 3 times. The pots were moderately watered at intervals of 3-4 days on need basis. Soil samples were randomly collected at 60 days after planting and at harvest from three pots in each treatment to determine cysts count and egg viability.

Field experiment

The field trial was planted in naturally PCN infested soils in a randomized complete block design (RCBD) with three replications and repeated once. The experimental plots were 3 m × 3 m in size, with plant spacing of 75 cm × 30 cm. The treatments were similar to those used in the screenhouse experiment and were applied at planting. Soil samples (300 g) were collected from each plot before

planting, at 60 days after planting and at harvest to determine cyst/300 g and egg counts in each cyst.

PCN cyst extraction. Cysts were extracted by the floatation method (Fenwick,1940) from the soil samples collected.

Egg viability determination. Three individual cysts extracted from each sample were placed in well of the 96 well plate having Nile blue stain (0.01 %) and incubated in the dark for 48 hrs. The cyst were then crushed gently with a sterile needle to release eggs and J2s. The contents of the three cysts in each of the three pots per treatment were then determined. Whereas the stained eggs were considered non-viable, unstained eggs were considered viable.

Data collection

Pest parameters

Cyst counts. Following extraction, cysts were picked and counted under a microscope at 40X magnification**.** At planting, the initial population (P_i) density of cyst per 300 g soil and egg viability per 3 cysts of each treatment was determined. The middle population (P_m) density of cyst per 300 g soil and egg viability per three cysts was determined in each of the three pots of each treatment at 60 days after planting in the screenhouse. At harvest time, the final population (P_f) densities of cyst and egg viability per three cysts was determined in each of the three pots of each treatment was evaluated.

Potato yield. Yield data were collected from only the field experiments. During harvesting, 3 inner rows, excluding the plants from the guard rows were considered for determination of fresh tuber yields. Tuber yields were further graded based on tuber diameter into ware (>55 mm), seeds (25-55 mm) and chats (<25 mm). The average tuber weight was determined from the weight of all the tubers in the middle row divided by the total number of tubers from the row.

6.3.6 Determination of active phyto-chemical constituents in ginger, garlic and Mexican sunflower

Qualitative and quantitative analysis of phytochemicals

The study was conducted at the University of Nairobi in the Department of Public Health Pharmacology and Toxicology Faculty of Veterinary Medicine laboratory in 2022. Using the standard laboratory procedures described by Bargah, 2015; Otieno *et al.*, 2016, tests were carried out to determine the presence of nematicidal phytochemical within the hexane extracts of ginger, garlic and Mexican sunflower.

Qualitative analysis of phytochemicals

Qualitative tests were carried out using a modified protocol as previously described by Otieno *et al.*, (2016) to determine the presence of saponins (foam test), phenols and tannins (ferric chloride test), flavonoids (alkaline reagent test), glycosides (Salkowski's test), steroids (Liebermann's test), alkaloids (Mayer's test) and terpenoids.

Quantitative analysis of phytochemicals

Quantitative determination of the detected secondary metabolites were carried out to determine their percentages in the hexane extracts of ginger, garlic and Mexican sunflower by the methods described by Tabe *et al.*(2019).

Quantitative analysis of phenol

The spectrophotometric method was used to determine the amount of phenolic extracts in ginger garlic and Mexican sunflower. Ten milligrams of each extracts were dissolved in ten milliliters of methanol. One (1) ml of this solution was transferred to ten milliliters volumetric flask, along with 2.5 ml of Folin Ciocalteau reagent and 2.0 of 7.5 % w/v sodium carbonate (Na_2CO_3). The mixture was then diluted to 10ml. A mixture of reagents and water was used as blank. Gallic acid was used as a standard. The sample was left for 30 minutes to develop color before measuring absorbance at 765nm. The percentage of phenols was determined using the following formula:

$$\text{Percentage phenols} = \frac{\text{Sample absorbance X Standard concentration}}{\text{Absorbance of standard}} \text{X100}$$

Quantitative analysis of Tannins

The quantity of tannins was determined by using the spectrophotometric method. Each extract measured 0.25 g was placed in conical flask and dissolved in 25 ml of distilled water and stirred for 1hr. The sample was filtered into a volumetric of 25 ml. Thereafter, 1.25 ml of Folins denis reagent and 2.5 ml of saturated Na_2CO_3 were added to the flask, which was then made up to the mark using distilled water. The absorbance was measured at 700nm win 10 min. Tannic acid was used as the standard. The formula described by Tabe (2019) to calculate the tannic acid content as follows:

$$\text{Percentage tannin} = \frac{\text{Sample absorbance X Standard concetration}}{\text{Absorbance of standard}} \text{X100}$$

Quantitative determination of flavonoids

The flavonoids content were determined according to modified method described by Otieno *et al.,* (2016). Ten milligram of each extract was dissolved in ten milliliters of methanol to make a solution with a concentration of 1 mg/ml. An aliquot of each extract was transferred to a ten milliliter volumetric flask containing four milliliters of distilled water. This was mixed with 0.3 ml of 5 % w/v sodium nitrite ($NaNO_2$). After five minutes, 0.3 ml of 10 % w/v aluminium chloride ($AlCl_3$) was added to the solution. After six minutes, 2 ml of 1M sodium hydroxide (NaOH) was added and made up to 10 ml with distilled water. The absorbance was measured at 510 nm. Distilled water served as a blank, while Rutin served as a standard.

$$\text{Percentage flavinoids} = \frac{\text{Sample absorbance X Standard concetration}}{\text{Absorbance of standard}} \text{X 100}$$

Quantitative Analysis of Alkaloids

The amount of alkaloid was determined using a modified method described by Otieno *et al.*, (2016). In 100 ml beaker, 50 g of 10 % acetic acid in ethanol was added to 0.625 of each sample extract and the mixture was allowed to stand for four hrs. The mixture was then concentrated to ¼ of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide (NH_4OH) was added until precipitation was complete. The mixture was then allowed to settle and the

supernatant discarded. The precipitate was then washed with 20 ml of 0.1 M of ammonium hydroxide (NH_4OH) and filtered. After that, the residue was transferred to a crucible and dried in the oven. The percentage alkaloid was calculated as follows:

$$\text{Percentage alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of the sample}} \text{X100}$$

Quantitative analysis of saponins

In a 250 cm^3 conical flask, 50 g of 20 % ethanol was added to 5 grams of each extracts. The mixture was then heated for 4 hours at 55 ^{0}C in a hot water bath with continuous stirring. This was then filtered and the residue re-extracted with another 20 % ethanol and heated at 55 ^{0}C for 4 hours with constant stirring. The extract was then evaporated at 40 ^{0}C in a water bath. Twenty milliliter of diethyl ether was added to the concentration in a 250 ml separating funnel and vigorously agitated. The ether layer was discarded and the aqueous layer was covered. This procedure was carried out twice. The extract was then extracted twice with 60 ml of n-butanol and then extracted twice with 10 g of 5 % w/v sodium chloride. The remaining solution was heated in water bath for 30 minutes after the sodium chloride layer removed. The solution was then transferred to a crucible and oven dried to a constant weight. The saponins content was calculated as follows:

$$\text{Percentage saponin} = \frac{\text{Weight of saponin}}{\text{Weight of the sample}} \text{X100}$$

Quantitative determination of terpenoids

Terpenoids were quantified using the method described by Otieno *et al.* (2016). In extracts 0.1g were taken and soaked in 9ml of absolute ethanol for 24 hrs. The mixture was then filtered through whatmann filter paper and 10 ml of petroleum ether was added to the filtrate. A separating funnel was used to separate the pet layer. The pet ether was allowed to dry until it reached a constant weight. Terpenoids were calculated as follows:

$$\text{Percentage terpenoids} = \frac{\text{Weight of terpenoids}}{\text{Weight of the sample}} \text{X100}$$

6.4 Data analysis

The Shapiro-Wilk test was used to determine the normality of the entire data set. Percent viable PCN eggs were computed using the following formula by Aileen and Devine, 2005:

$$\text{Percentage viable eggs} = \frac{\text{Viable eggs}}{\text{Viable eggs} + \text{non} - \text{viable eggs}} \text{X100}$$

On juvenile mortality, data collected on live and dead J2s were used to compute the mean percentage juvenile mortality using the formula by Fatemy (2018).

$$\text{Percentage juvenile mortality} = \frac{\text{No. of nonviable juveniles}}{\text{No. of viable juveniles} + \text{non} - \text{viable juveniles}}$$

Potato cyst nematode cyst and egg reproduction index were expressed as ($RI = P_f/P_i$) according to Van Den Berg and Rossing (2005). Before performing analysis of variance (ANOVA), the data on cyst and egg counts were normalized using square root or log (x+1) transformation (Gomez, 1984) was conducted. Data were subjected to ANOVA using the Statistical Analysis Software (version.8.2, SAS). Least significant differences (LSD) tests were used to separate treatment means at 5 % level of significance. The 20, 50 and 80 % lethal concentration at 95 % confidence limit (CL), regression equation and percentage coefficient of determination (R^2) were computed using Probit analysis (Finney, 1971).

6.5 Results

6.5.1 *In-vitro* assay

Experiment 1. Determination of the hatchability and viability of PCN eggs when exposed to plant extracts at different times

Effect of the plant extracts on the PCN egg viability

The microscopic images of the eggs observed with Nile blue stain indicated that the eggs were non-viable, the unstained eggs were recorded as viable eggs and empty shells were recorded as hatched eggs and also disintegrated cysts was also observed (Figure 6.1).

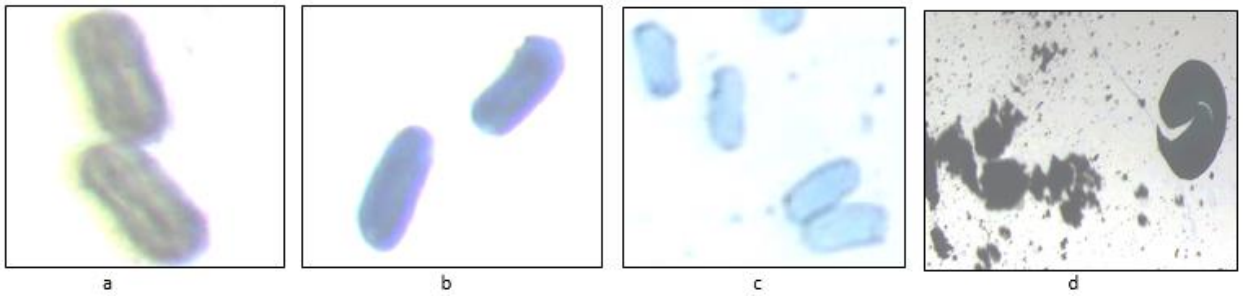

Figure 6.1.Microscopic images of potato cyst nematode eggs; (a) Viable eggs (unstained eggs), (b) Non-viable eggs (Nile blue stained eggs), and (c) Hatched eggs (egg shells) d) Disintegrated eggs at a 40X objective on a compound microscope

Plant extracts, solvents and time of exposure were highly significant in loss of egg viability (Table 6.2). All the plant extracts tested were found to exhibit some level of inhibition towards egg hatch and viability of the potato cyst nematode. The percentage number of non-viable and viable eggs for each plant extract after 72 hrs after treatment is presented in Table 6.2. In both experiments, all the plant extracts exhibited a low percentage of viable eggs (11.8 % to 39.1 %) compared to potato root diffusate which had 72.4 % to 78.1 % viable eggs (Table 6.3). Exposure of PCN eggs to plant extracts led to a significant ($P<0.05$) decrease of viable eggs/cyst ranging from 18.3 to 43.7 compared to PRD which had 79.4 and 108.1 viable eggs/cyst in experiment 1 and 2, respectively (Table 6.3).

Table 6.2. Effects of plant extracts, exposure time and solvent on potato cyst nematode non-viable eggs

Sources of variation	df	Experiment 1.			Experiment 2		
		Sum of squares	F	P	Sum of squares	F	P
Treatments	11	343838	4.66	<.001	315032	4.05	<.001
Hours of incubation	2	7086	0.58	<.001	14139	1.09	<.001
Solvent	3	377744	20.50	<.001	342728	17.62	<.001

Among the ten plant extracts evaluated, only three (ginger, Mexican sunflower and garlic) showed a high and consistent number of non-viable egg counts. In experiment 1. Ginger had 112.2 non-viable eggs/cyst while in experiments 2 there were 120.2 non-viable eggs/cyst. Mexican sunflower had 151.9 non-viable eggs/cyst in experiment 1 and 144.9 non-viable eggs/cyst in experiment 2. Garlic had 138.1 non-viable eggs/cyst in experiment 1 and 134.0 non-viable eggs/cyst in

experiment 2. In comparison to neem the positive control, both garlic and Mexican sunflower had a high number of non-viable eggs. The PRD treatment had the lowest loss of egg viability per cyst with 30.3 and 30.2 non-viable eggs/ cyst for experiment 1 and 2, respectively (Table 6.3).

Effect of exposure time of the different plant extracts on loss of egg viability

The *in-vitro* assay showed that all plant extracts were effective in reducing potato cyst nematode eggs viability as compared to PRD when subjected to the three-time regimes post treatment (Figure 6.2, 6.3). Generally, the loss of egg viability increased with increase in exposure time. Treatment exposure after 72 hrs on potato cyst nematode had the highest loss of egg viability.

Mexican sunflower, garlic and ginger extracts were highly significant ($p \leq 0.05$) in inducing loss of egg viability compared to the other plant extracts and potato root diffusate at all the three exposure times. At 72 hrs, Mexican sunflower extracts resulted in 85.1 % and 82.3 % non-viable

Table 6.3. Effect of different plant extracts on potato cyst nematode egg viability

Plant extracts	Experiment 1			Experiment 2		
	Non-viable eggs/cyst	Viable eggs/cyst	% Viable eggs	Non-viable eggs/cyst	Viable eggs/cyst	% Viable eggs
Mexican sunflower	151.9(2.1efg)	25.8 (1.2de)	14.5	144.9(2.1de)	29.9 (1.3d)	17.1
Garlic	138.1 (2.0e)	18.5 (1.1f)	11.8	134.0(2.0cd)	28.8 (1.3d)	17.7
Neem (Positive control)	137.6 (2.0ef)	23.6(1.0def)	14.6	124.6(2.0cd)	29.2 (1.3d)	19.0
Ginger	112.2 (1.9de)	22.8(1.2def)	16.9	120.2(2.0cd)	25.4 (1.3d)	17.4
Mexican marigold	86.2 (1.8cd)	27.0(1.3d)	23.9	85.9 (1.9c)	43.7 (1.5)	33.7
Spring onion	68.1 (1.7bc)	26.5 (1.2d)	28.0	71.1 (1.7b)	30.6 (1.3d)	30.1
Sodom apple	67.3 (1.7bc)	37.3 (1.2c)	35.7	64.09 (1.7b)	25.3 (1.2d)	28.3
Eucalyptus leaves	66.9 (1.7bc)	18.8 (1.1f)	21.9	67.9 (1.7b)	20.2 (1.2d)	22.9
Eucalyptus bark	64.4 (1.6b)	31.2 (1.4d)	32.6	64.8 (1.6b)	28.9 (1.2d)	30.8
Green tea leaves	62.5 (1.6b)	20.5(1.1def)	24.7	59.3 (1.6b)	38.0 (1.4c)	39.1
Onion bulb	59.9 (1.6b)	19.9 (1.1ef)	24.9	59.4 (1.6b)	18.3 (1.1e)	23.6
[1]PRD(Negative control)	30.3 (1.4a)	79.4 (1.7a)	72.4	30.3 (1.4a)	108.1(2.0a)	78.1
$LSD_{0.05}$	21.0 (0.2)	6.7 (0.2)	7.79	22.6(0.15)	7.8(0.08)	7.79

[1]PRD-Potato root diffusate. Figures in parenthesis represent transformed data; all data are means of three replicates; Means followed by the same letter within the columns are not significantly different at ($p \leq 0.05$) according to Fisher's protected least significant difference test.

eggs/ cyst in experiment 1 and 2, respectively. While garlic extracts at 72 hrs had 80 % and 88 % non-viable egg/ cyst in experiment 1 and 2, respectively, whereas those with ginger had 85.1 % and 86.6 % of non-viable egg/ cyst in experiment 1 and 2, respectively at 72 hrs (Figures 6.2 and 6.3). Amongst the plant extracts, Mexican sunflower activity led to 80.2 % and 73.4 % non-viable eggs/cyst in experiment 1 and 2, respectively at 48 hrs. At 48 hrs, garlic induced percentage loss of egg viability per cyst of 79.5% and 68.3% in experiment 1 and 2, respectively. Ginger activity as associated with 79.5 % and 64.4 % non-viable eggs/cyst in experiment 1 and 2, respectively at 48 hrs (Figures 6.2 and 6.3). After 24 hrs of exposure of potato cyst nematode eggs to plant extracts, a higher nematicidal activity was recorded in Mexican sunflower having percentage loss of egg viability per cyst of 75.2 % and 60 % in experiment 1 and 2, respectively, followed by garlic having 69.9 and 64.9 % non-viable eggs/cyst in experiment 1 and 2, respectively. Ginger had 69.6 % and 61.5 % loss of egg viability per cyst in both experiments respectively (Figures 6.2 and 6.3)

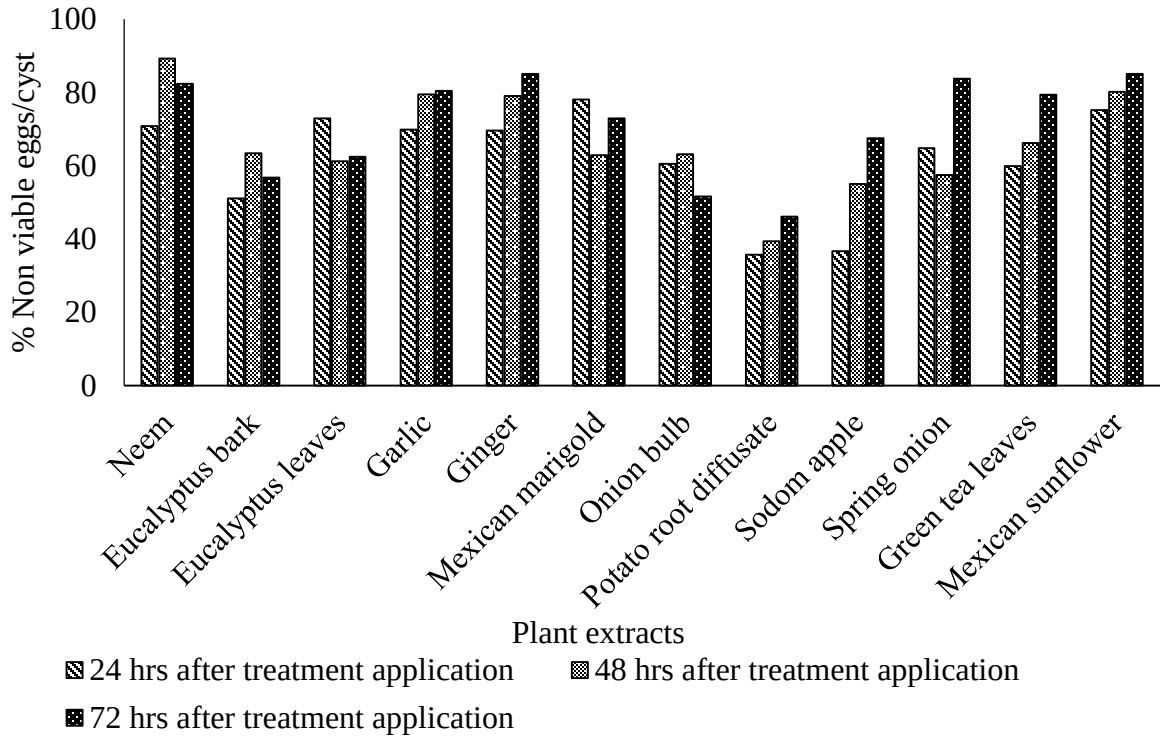

Figure 6.2. Effect of incubation period of the plant extracts on loss of egg viability of the potato cyst nematodes in experiment 1

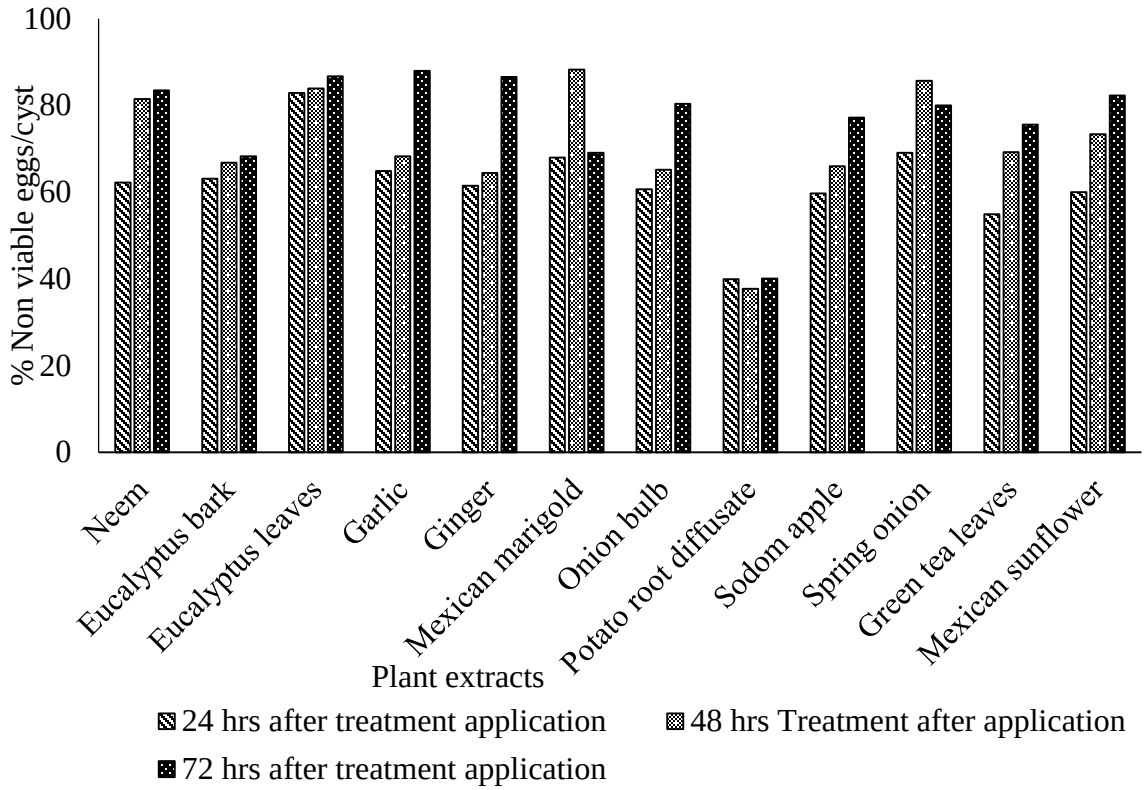

Figure 6.3. Effect of incubation period of the plant extracts on loss of egg viability of the potato cyst nematodes in experiment 2

Effect of exposure time of the different plant extracts on percentage egg hatch

The results showed that when PCN eggs were exposed to plant extracts at different times (24, 48 and 72 hrs), egg hatch of infective juveniles occurred (Figures 6.4 and 6.5). After all the three exposure times. PCN egg hatch was significantly higher ($p \leq 0.05$) in the PRD control than in all other treatments (Figure 6.4 and Figure 6.5). In experiment 1, among the plant extracts, Sodom apple had the least percentage PCN egg hatch/cyst (3.0 %) after 24 hrs of exposure, while at 48 and 72 hrs of treatment exposure, garlic had the least percentage egg hatch/cyst of 5.0 % and 2.5 %, respectively (Figure 6.4). In experiment 2, the lowest percentage of egg hatch inhibition/cyst (4.5 %) was observed in the spring onion extract after 24 hrs of exposure (Figure 6.5). After 48 and 72 hrs of treatment exposure, garlic had the least percentage egg hatch of 3.3 % and 2.4 %, respectively (Figure 6.5).

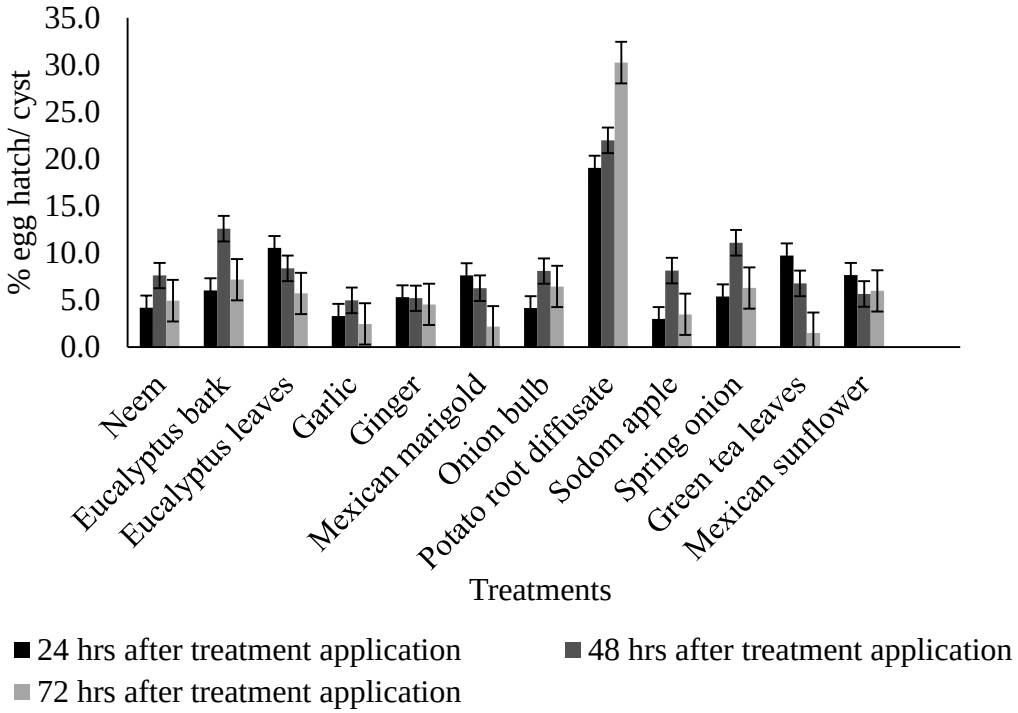

Figure 6.4. Effect of plant extracts on percentage egg hatch of the potato cyst nematodes after 24, 48 and 72 hrs of exposure in experiment 1

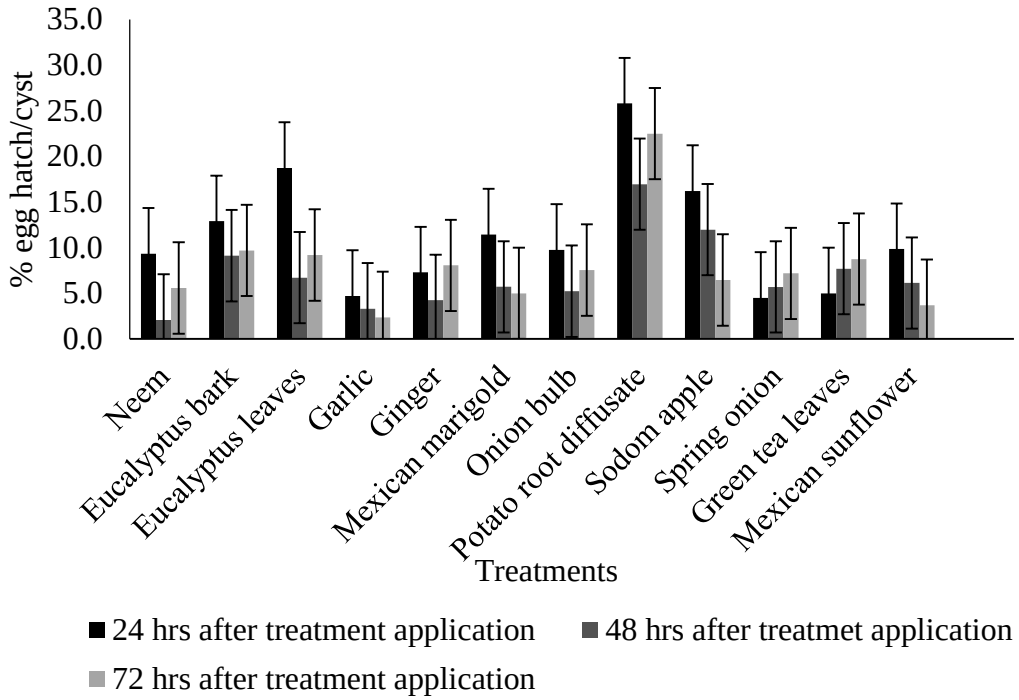

Figure 6.5. Effects of plant extracts on percentage egg hatch of the potato cyst nematode after 24, 8 and 72 hrs of exposure experiment 2

Effect of different solvents extracts on loss of egg viability

Plant extracts from ethyl acetate, hexane and methanol solvents caused higher loss of egg viability compared to that of water extracts (Figures 6.6 and 6.7). However, when compared to other solvents hexane extracts had significantly higher and consistent mean loss of egg viability in the two experiments (Figures 6 and 7). Hexane extracts of Mexican sunflower resulted in a significantly higher loss of egg viability having 93 % and 89.2 % non-viable eggs/cyst in experiment 1 and 2, respectively compared to other plant extracts. This was followed by garlic which had 89.5 % and 86.3 %, and then ginger 86.8 % and 85.9 % non-viable eggs/cyst in experiment 1 and 2, respectively (Figures 6.6 and 6.7).

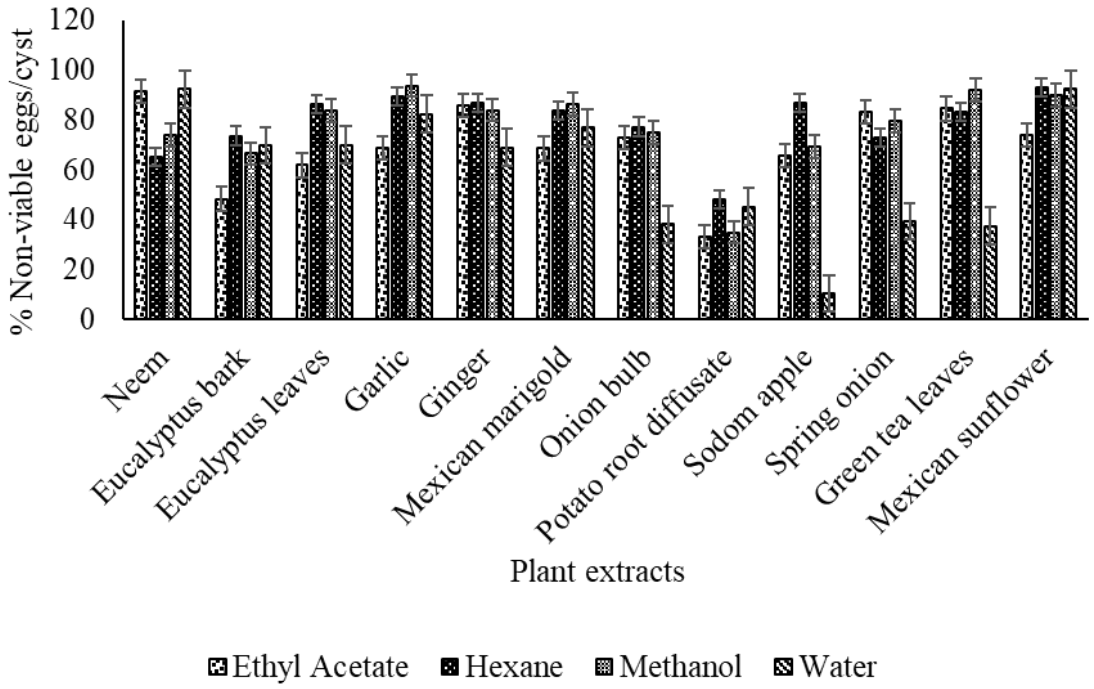

Figure 6.6. Effect of solvent extracts on loss of egg viability of potato cyst nematodes in experiment 1

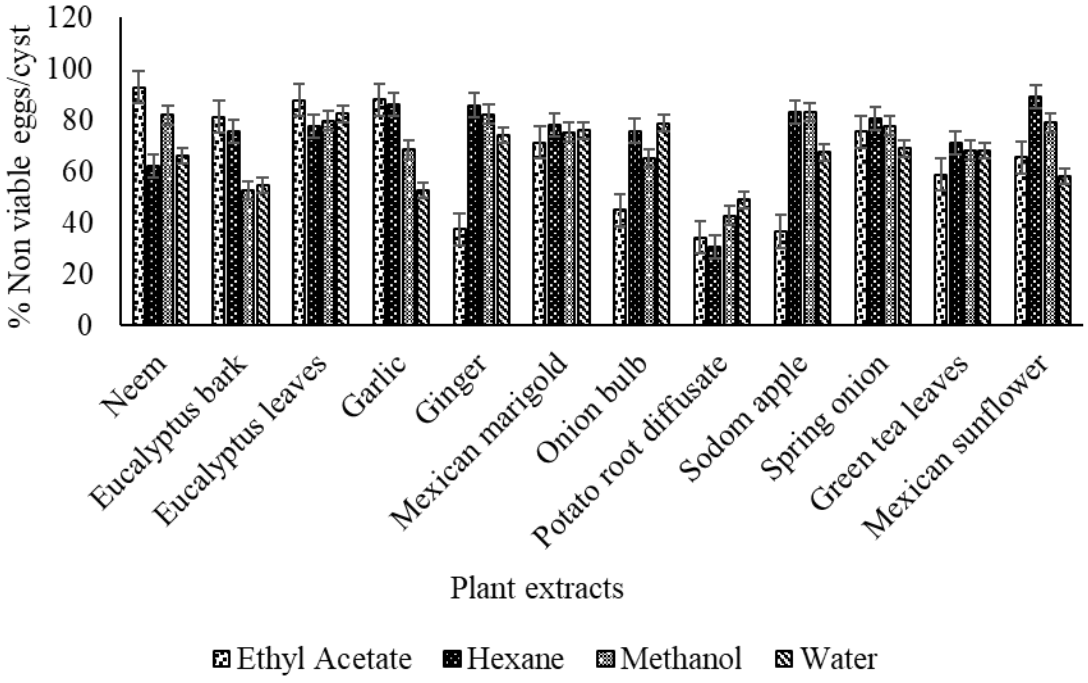

Figure 6.7. Effect of solvent extracts on loss of egg viability of potato cyst nematodes in experiment 2

Experiment 2. Determining PCN second stage juvenile mortality when exposed to different plant extracts at different time intervals

Effect of solvents and plant extracts on PCN second stage juvenile mortality of potato cyst

All the four solvents (ethyl acetate, hexane, methanol and water) had no significant ($p \leq 0.05$) influence on juvenile mortality. All the ten plant extracts induced juvenile mortality. Ginger induced the highest juvenile mortality of 68.9 % and 63.3 % in experiment 1 and 2 respectively, followed by treatments with Mexican sunflower with 67.9 % and 60.1 % dead juveniles then garlic with 64.3 % and 60.8 % dead juveniles in experiment 1 and 2, respectively (Table 6.4). Water alone had a few J2s of potato cyst nematodes (25.7 % and 25.2 %) compared to the plant extracts (Table 6.4). Potato root diffusate had the least juvenile mortality in both experiments (15.5 % and 17.7 %) (Table 6.4). An illustration of live juveniles when J2s were subjected to control checks and dead juveniles when J2s were subjected to plant extracts treatments is as shown in Figure 6.8.

Table 6.4. Effect of different plant extracts on mortality of second stage juveniles of potato cyst nematodes

Treatments	Experiment 1		Experiment2	
	Dead Juveniles/2ml (%)	Active juveniles/2ml (%)	Dead juveniles/2ml (%)	Active juveniles/2ml (%)
Neem (Positive control)	69.4e	30.6a	65.1d	34.9a
Ginger	68.9e	31.1a	63.3d	36.7a
Mexican sunflower	67.9e	32.1a	60.1d	39.9a
Garlic	64.3e	35.7a	60.8d	39.2a
Onion bulb	47.4d	52.6b	43.6c	56.4b
Spring onion	44.1cd	55.9bc	43.3c	56.7b
Eucalyptus leaves	43.3cd	56.7bc	43.4c	56.6b
Mexican Marigold	42.8cd	57.7bc	44.5c	55.5b
Sodom apple	41.9cd	58.1bc	42.4c	57.6b
Eucalyptus Bark	41.7cd	58.3bc	41.3c	58.7b
Green tea leaves	39.5c	60.5c	42.3c	57.7b
Water (negative control)	25.72b	74.28d	25.3b	74.75c
[1]PRD (negative control)	15.5a	84.5e	17.7a	82.3d
$LSD_{0.05}$	6.51	6.518	6.37	6.37

[1]PRD-Potato root diffusate, LSD-Least significant differences is used to separate treatment means at 5 % level of significance

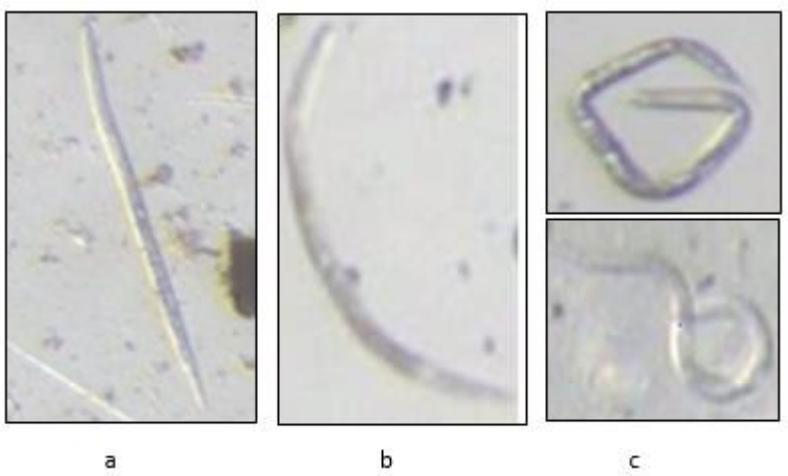

Figure 6.8. Characteristic shapes of dead juveniles: a. straight (I-shape), b. slightly bent (banana-shape), and c. curled (∞ -shape) at a 40 X objective on a compound microscope

Determination of second stage juvenile mortality when exposed to different plant extracts at different time intervals

The bioassay showed that all the plant extracts positively influenced the mortality of the potato cyst nematode J2s when applied at 24, 48 and 72 hrs (Figures 9 and 10). Juvenile mortality rate was significantly influenced ($p \leq 0.05$) by exposure time positively. In both experiments, there juveniles mortality ranged from 36.0 % to 73.0 % when exposed to the plant extracts in all the three time regimes compared to the untreated check PRD. In experiment 1, among the 10 plant extracts tested, garlic, Mexican sunflower and ginger had significantly high ($p \leq 0.05$) juvenile mortalities (68.8 %, 72.6 % and 73.0 %, respectively) after 72 hrs of treatment exposure compared to other plant extracts (Figures 6.9 and 6.10). In experiment 2, after 72 hrs of treatment exposure garlic, Mexican sunflower and ginger had significantly high ($p \leq 0.05$) juvenile mortalities of 64.5 %, 64.9 % and 70.2 %. These plant extracts (garlic, Mexican sunflower and ginger) showed a similar efficacy after 24 and 48 hrs of treatment exposure in both experiments. The potato root diffusate treatment had the lowest juvenile mortality in all the three time intervals in both experiments.

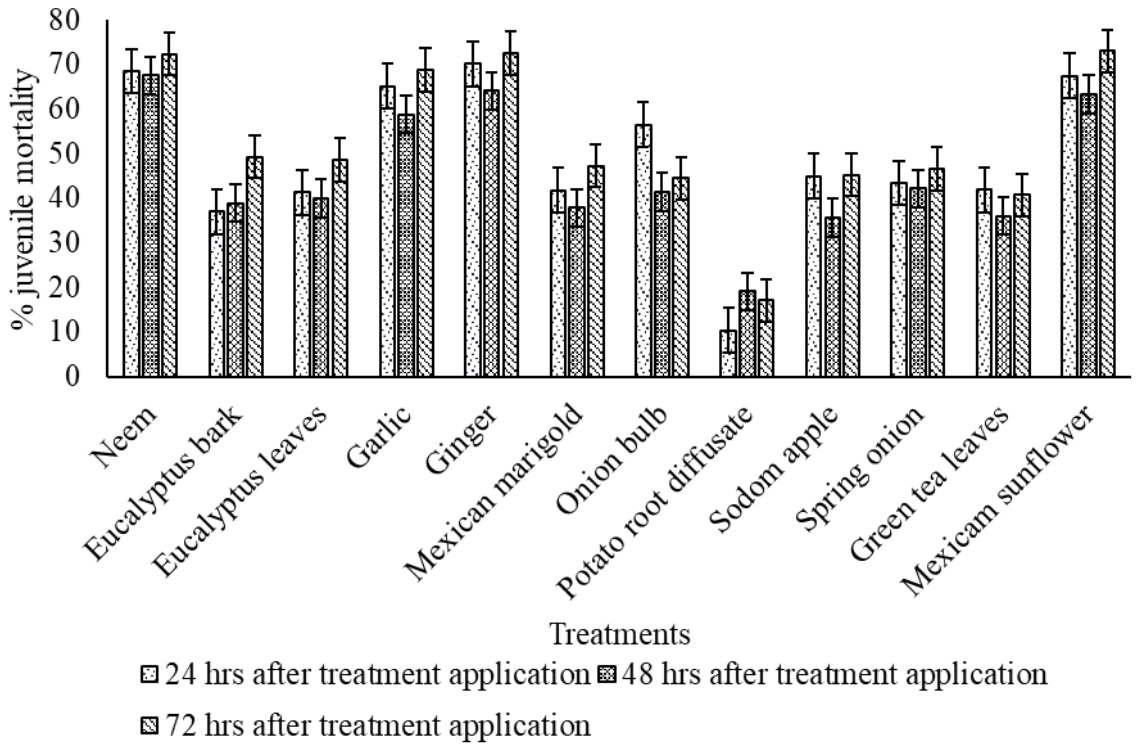

Figure 6.9. Percentage juvenile mortality in plant extracts at different exposure times in experiment 1

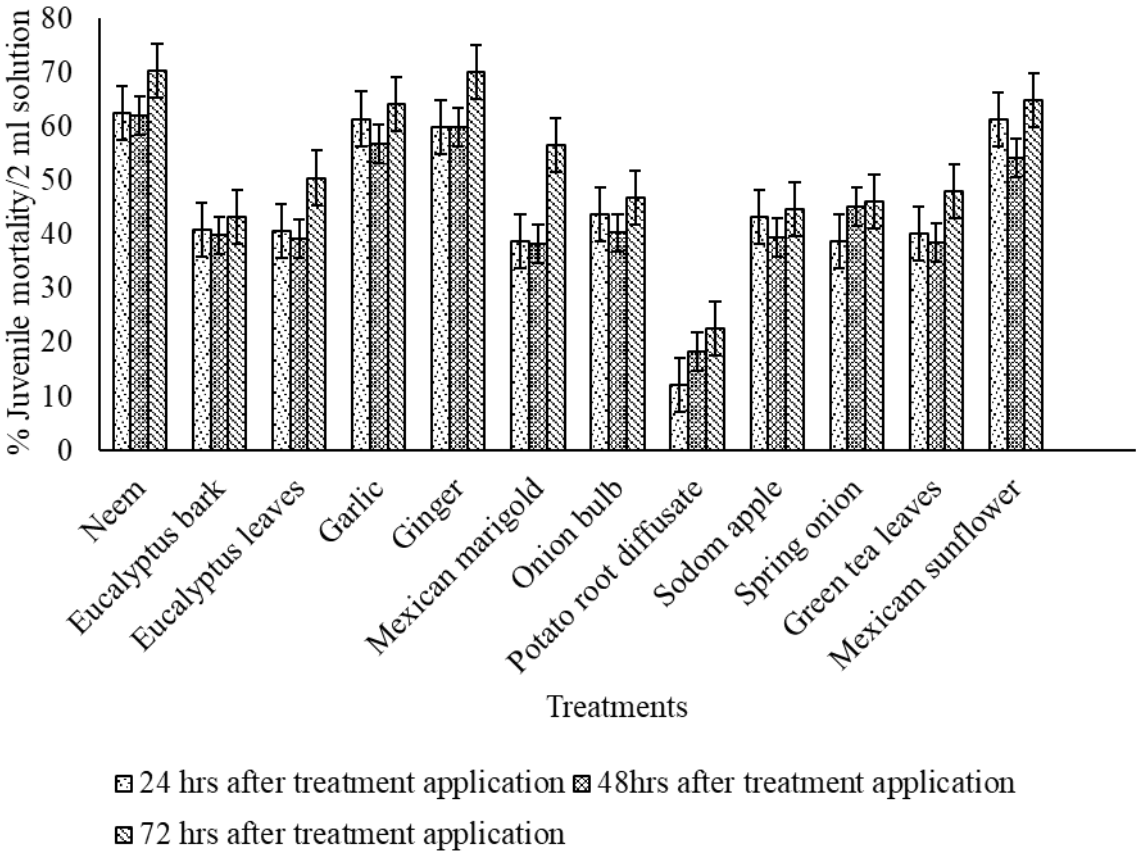

Figure 6.10. Percentage mortality of juveniles in plant extracts at different exposure times in experiment 2

6.5.2 Screenhouse experiment

Effect of plant extracts on PCN population in the screenhouse

Total PCN reproduction index (RI) was significantly reduced in all the tested treatments with the reproduction index ranging from 0.50-0.70 over the seasons. Generally, the final PCN population density (P_f) was significantly reduced having 2.7 to 13.3 cysts /300 g soil by the botanical extracts when compared to the untreated control having 92 cysts / 300 g soil during the short rains. Similarly, the final PCN population density was significantly reduced ranging from 4.3 to 12.3 cysts /300 g soil by the botanical extracts when compared to the untreated control having 88 cysts / 300 g soil during long rains season. During short and long rains ginger extract, applied at a concentration of 100 mg/ml, had the lowest reproduction index 0.10 and 0.17, respectively (Table 6.5). The highest % reduction of cysts over the control (96.9 and 95.4 % during the short and long rains, respectively) was achieved when the ginger extract was applied at 100 mg/l (Table 6.5).

All the treatments reduced the population density of PCN eggs compared to the control during the short and long rains, respectively. The highest RI values of 2.38 and 1.43 were observed in the untreated plants during the short and long rains, respectively (Figure 6.11). Among the extracts, ginger extract at the concentration of 50 mg/l caused the greatest decrease in the number of newly formed eggs having a reproductive index of 0.27 and 0.29 during the short and long rain seasons, respectively. Among the extracts, Mexican sunflower at the concentration of 50 mg/l had the highest reproductive index was 0.55 and 0.53 during short and long rains, respectively.

6.5.3 Field experiment

Effect of extracts on yield

A significant increase in potato yield was observed in all plots that were treated with the test extracts compared to the control (Table 6.6). The yield increase in plots treated with ginger extracts, at a concentration of 100 mg/ml was 112.2 % an equivalent of 31.47 t ha^{-1} and 80.6 % an equivalent of 18.96 t ha^{-1} during the short and long rains, respectively (Table 6.6).

Effect of extracts on PCN population density

The final population (P_f) density of cysts showed that the plant extracts significantly ($p \leq 0.05$) reduced the cyst density in the field when compared with the control (Table 6.7). Ginger, garlic and Mexican sunflower showed different levels of PCN cyst and egg reduction. Among the extracts, the plants that received ginger extracts at the concentration of 75 mg/ml showed the highest percent cyst reduction of 71.4 % and 75.6 % over the plants that did not receive extracts.

Effect of extracts on PCN Reproduction index (RI)

The PCN RI was significantly reduced in all the tested treatments with the reproduction index ranging from 0.50-0.70 over the seasons (Table 6.7). Plants that received ginger extract at a concentration of 100 mg/ml had the lowest reproduction index of 0.50 followed by ginger extract at concentration of 75mg/l having 0.53 RI. Similarly, plants treated with garlic at concentration of 100 mg/l, showed reproduction index of 0.53. Amongst the 3 extracts, garlic and Mexican sunflower at concentration of 25 mg/ml showed the highest reproduction index of 0.63 during the.

Table 6.5. Effects of varying concentrations of ginger, garlic and Mexican sunflower extracts on potato cyst nematodes on potato cultivar Shangi

Extracts		Short rains Cysts count/300 g soil					Long rains Cysts count/300 g soil			%Reduction in P_f over Control	RI
	Conc. (mg/ml)	P_i	P_m	P_f	% P_f Red. over Control	RI	Pi	P_m	P_f		
Ginger	25	30	23.0(2.43)bcd	6.3(0.8)bcd	92.8	0.20	30	26.3(0.62)b	12.3(0.66)b	86.7	0.40
	50	30	17.0(2.83)bc	8.3(0.7)bcde	90.6	0.30	30	16.0(0.46)bc	6.7(0.70)bc	92.7	0.23
	75	30	17.0(3.26)b	10.7(0.7)bcde	87.8	0.37	30	14.0(0.43)bc	8.7(0.76)bc	90.6	0.30
	100	30	9.0(0.93)de	2.7(0.53)e	96.9	0.10	30	22.3(0.53)c	4.3(0.23)bc	95.4	0.17
	25	30	16.3(2.63)bc	7.3(0.7)bcde	91.7	0.23	30	8.6(0.38)bc	7.3(0.70)bc	92.1	0.27
Mexican	50	30	16.0(1.53)cde	3.7(0.7)bcde	95.8	0.10	30	15.7(0.44)bc	10.7(0.43)bc	88.4	0.33
sunflow	75	30	20.7(3.50)b	13.3(0.8)b	84.9	0.47	30	8.00(0.37)bc	4.3(0.76)bc	95.3	0.17
er	100	30	23.3(2.10)bcde	4.7(0.9)bc	94.7	0.13	30	17.7(0.48)bc	4.3(0.63)bc	95.3	0.18
	25	30	19.0(3.10)b	10.0(0.7)bcde	88.6	0.33	30	18.0(050)bc	10.7(0.76)bc	88.4	0.36
Garlic	50	30	20.3(2.93)bc	9.0(0.8)bcde	89.8	0.30	30	21.7(0.52)bc	11.3(0.73)bc	87.8	0.40
	75	30	23.7(3.40)b	11.7(0.9)b	86.7	0.40	30	22.3(0.54)bc	9.3(0.76)bc	89.9	0.30
	100	30	12.0(1.86)bcde	6.0(0.5)de	93.2	0.20	30	17.0(0.49)bc	8.7(0.46)bc	90.6	0.30
Oxamyl	8l/ha	30	12.3(0.46)e	0.7(0.6)cde	99.2	0.03	30	7.7(0.28)c	2.7(0.16)c	97.1	0.10
Azadira	6l/ha	30	19.3(3.00)bc	9.3(0.8)		0.30	30	13.3(0.40)bc	8.0(0.73)bc		0.27
chtin					89.4					91.3	
Control	-	30	55.0(9.33)a	88.0(1.4)a	0.0	2.93	30	56.3(1.7)a	92.3(1.33)a	0.0	3.10
$LSD_{0.05}$			1.64	0.27				0.32	0.35		
CV (%)			21.3	21.3				34.2	32.2		

Data in parenthesis are square root transformed

Conc. = concentration, Means within the same column with a common letter are not significantly different ($p \leq 0.05$)

P_i: population initial, P_m: Middle population, P_f: Final population Reproduction index final $RI=P_f/P_i$

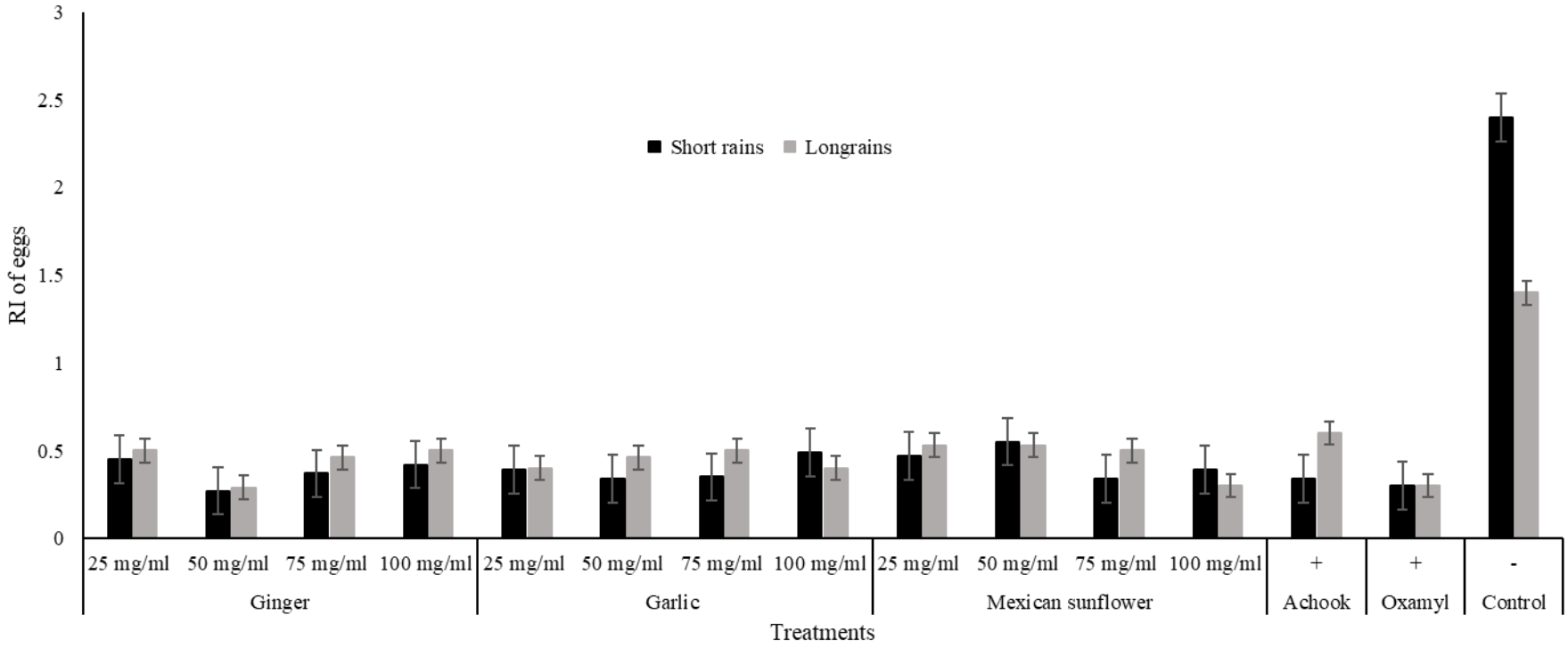

Figure 6.11. Effects of different concentrations of plant extracts on the reproduction rate of PCN eggs under screenhouse conditions

Table 6.6. Effects of plant extracts on potato yield in potato cyst nematode infested fields

Treatments	Concentration	Yield t ha^{-1} (Mean ± SE)									
		Short rains season 2021					Long rains season 2021				
		Ware	Seed	Chatts	Yield	% yield increase over control	ware	seed	Chatts	Total yield	% yield increase over control
Ginger	25	9.1±1.53abc	10.67 ±0.89bc	0.78±0.07b	20.52±2.33b	38.4	8.47±2.95ab	8.33±0.83	0.33±0.23	17.17±4.01abcd	63.5
	50	10.9±2.88ab	10.86±2.02bc	1.17±0.21b	22.89±4.93ab	54.3	12.16±1.83a	5.83±1.23	0.46±0.3	18.47±2.77ab	75.9
	75	8.7±1.55abc	11.15 ±0.88bc	1.06±0.25b	20.89±2.48a	40.9	5.67±1.48bc	5.33±0.53	1.03±0.08	12.07±0.86bcd	15.7
	100	15.0±2.470a	10.91±0.38bc	5.56±0.16a	31.47±2.69a	112.2	11.7±1.87bc	6.29±0.95	0.97±0.28	18.96±2.86abcd	80.6
Garlic	25	9.5±0.88abc	10.98 ±o.57bc	1.39±0.230b	21.91±1.43b	47.7	6.23±1.85bc	6.83±2.10	036±0.38	13.40±4.33abcd	27.6
	50	6.7±1.51bc	10.57±0.29bc	1.11±1.11ab	18.41±1.24b	24.1	7.23±0.95ab	6.50±1.03	0.43±0.03	14.20±1.95abcd	35.2
	75	9.8±1.42ab	14.05 ±0.67ab	1.11±0.35b	25.05±1.81ab	68.9	8.23±3.22ab	6.17±5.24	0.67±0.51	15.07±8.97abcd	43.5
	100	10.7±2.71ab	16.4 ±1.31a	1.89±0.09b	28.98±3.12a	95.4	7.43±2.72ab	5.87±3.34	0.53±0.12	13.80±6.19abcd	31.4
Mexican sunflower	25	8.2±1.90 abc	9.95±1.03bc	1.23±0.22a b	19.38±2.80b	30.7	5.20±2.63bc	5.03±1.00	0.53±0.49	10.70±1.13bcd	1.9
	50	7.3±1.45abc	12.29±0.34abc	1.67±0.38ab	21.31±1.33c	43.7	4.53±0.08bc	7.27±1.65	0.83±0.37	12.40±2.57bcd	18.1
	75	8.9±1.11abc	11.87 ±0.73abc	1.38±0.23b	22.19±1.06b	49.6	4.53±1.29bc	6.43±1.65	0.70±0.36	11.70±2.57bcd	11.4
	100	9.2±1.02abc	8.00 ±0.89b	0.84±0.27b	18.07±1.43b	21.8	8.53±0.62 ab	8.50±0.52	0.40±0.36	17.40±0.47abc	65.7
Achook	6l/ha	10.3±2.200abc	10.14 ±1.588bc	1.23±1.130b	21.71±3.182c	46.4	11.7±0.83a	8.20±0.90	0.50±0.25	20.40±1.98a	94.3
Oxamyl	8l/ha	5.6±2.27bc	12.60±1.39abc	1.26±0.08a	19.44±3.58b	31.1	8.93±2.70ab	5.97±1.35	0.27±0.22	15.10±4.27abcd	43.8
Control	-	3.3±2.200c	2.55±1.588d	8.98±1.130a	14.83±3.182c	0	1.37±0.91c	1.56±0.39	5.57±0.74	10.5±0.22e	0
$LSD_{0.05}$		6.37	4.603	3.275	9.220		5.32	2.81	0.65	7.16	
CV (%)		42.87	25.32	29.58	25.91		31.2	31.2	31.2	31.2	

Data in parenthesis are square root transformed

Conc.: concentration, Means within the same column with a common letter are not significantly different ($p \leq 0.05$),

P_i: population initial, P_m: Middle population, P_f: Final population Reproduction index final $RI=P_f/P_i$

short rains season while Mexican sunflower had the highest reproductive index of 0.76 during the long rains respectively.

Lethal dose. Based on the LC_{20}, LC_{50} and LC_{80} values, aqueous extracts of ginger, garlic and Mexican sunflower showed higher nematostatic effects against PCN cysts than untreated control Ginger extracts were highly toxic to PCN cysts with the LC_{20}, LC_{50} and LC_{80} values at 20.67, 42.94 and 76.67 mg /ml, respectively. This was followed by garlic with LC_{20}, LC_{50} and LC_{80} values at 43.04, 79.57 and 116.09 mg ml^{-1}, respectively. Mexican sunflower had the least toxic effects against PCN cysts with the corresponding LC_{20}, LC_{50} and LC_{80} values of 56.10, 109.0 and 192 mg ml^{-1} (Table 6.8).

Table 6.7. Effects of concentration of plant extracts on potato cyst nematode population dynamics under field conditions

Extracts	Conc. ($mgml^{-1}$)	Short rains					Long rains				
		Cysts count/300 g soil			% Reduction in PCN population over Control	RI_f	Cysts count/300 g soil			% Reduction in PCN population over Control	RI_f
		P_i	P_m	P_f			P_i	P_m	P_f		
Ginger	25	54.2(7.4)a	50.3(7.0)b	32.3(5.6)bc	70.4	0.57	51.0(7.03)de	40.0(6.2)cd	31.3(5.5)cd	71.8	0.56
	50	69.3(8.1)a	54.7(7.2)b	36.0(5.9)bc	70.3	0.57	56.3(7.30)bcde	47.7(6.7)bcd	35.7(5.7)cd	71.9	0.56
	75	67.3(8.1)a	44.3(6.6)b	34.7(5.9)bc	71.4	0.53	54.7(7.37)bcde	41.3(6.3)cd	31.0(5.4)cd	75.6	0.53
	100	81.0(8.9) a	68.0(8.2)b	40.0(6.3)bc	67.0	0.50	79.7(8.9)abc	60.0(7.7)bcd	44.0(6.6)bcd	65.4	0.53
Mexican sunflower	25	68.7(8.2)a	60.3(7.7)b	40.7(6.4)bc	66.4	0.63	71.3(8.4)abcde	60.7(7.7)bcd	50.7(7.0)bcd	60.1	0.70
	50	78.0(8.8)a	65.3(8.0)b	46.0(6.8)bc	62.1	0.60	75.0(8.63)abcd	57.3(7.5)bcd	44.7(6.6)bcd	64.8	0.60
	75	84.7(9.1)a	68.0(8.1)b	42.0(6.5)bc	65.4	0.57	81.0(8.9)abc	62.7(7.5)abcd	43.3(6.5)bcd	65.9	0.57
	100	82.7(9.1)a	59.3(7.6)b	45.0(6.7)bc	62.9	0.57	88.7(9.4)a	68.0(8.2)a	47.7(6.9)bcd	62.4	0.53
Garlic	25	80.0(8.9)a	68.7(8.3)b	42.6(6.5)bc	64.9	0.63	80.3(8.9)abc	70.6(8.4)ab	61.0(7.7)b	51.9	0.76
	50	79.3(8.90a	67.3(8.1)b	50.7(7.0)bc	58.2	0.60	76.3(8.7)abcd	62.3(7.8)abcd	50.7(7.1)bcd	60.1	0.67
	75	84.7 (9.1)a	64.3(7.9)b	48.0(6.7)bc	60.4	0.57	84.0(9.2)ab	73.3(8.6)ab	54.0(7.3)bc	57.5	0.63
	100	81.3(9.0)a	72.0(8.4)ab	53.7(7.3)b	55.7	0.57	73.3(8.50)abcde	53.6(7.3)bcd	40.7(6.4)bcd	67.9	0.53
Oxamyl	8l/ha	64.0(7.9)a	55.0(7.3)b	36.7(6.0)bc	69.7	0.60	45.7(6.7)e	38.0(6.2)d	29.3(5.3)cd	76.9	0.67
Achook	6l/ha	67.7(8.2)a	61.7(7.8)b	34.7(5.7)bc	71.4	0.50	85.7(9.2)ab	66.0(8.1)abc	40.7(6.3)bcd	67.9	0.47
Control	-	54.3(7.3)a	98.7(9.9)a	121.3(11.0a	0.0	2.23	58.0(7.5)bcde	93.3(9.6)a	127.0(11.2)a	0	2.3
$LSD_{0.05}$		1.82	1.87	1.51			1.84	1.60	2.0		
CV (%)		12.90	14.18	13.48			13.22	14.73	17.6		

Data in parenthesis are square root transformed

Means within the same column with a common letter are not significantly different ($p \leq 0.05$)

P_i: population initial, P_m: Middle population, P_f: Final population Reproduction index final $RI=P_f/P_i$

Table 6.8. Plant extracts doses required to kill PCN extracted from soil treated with different concentrations of plant extracts

Plant extracts	Lethal Concentrations in mg ml^{-1} (95 % CL)			Regression equation	R^2 (%)
	LC_{20}	LC_{50}	LC_{80}		
Ginger	20.67	42.94	76.67	y = 0.03x+3.54	96.04
Garlic	43.04	79.57	116.09	Y=0.023x+3.17	89.43
Mexican sunflower	56.10	109.0	192.	y = 0.01x + 3.91	57.48

CL: Confidence limits, LC: Lethal concentrations

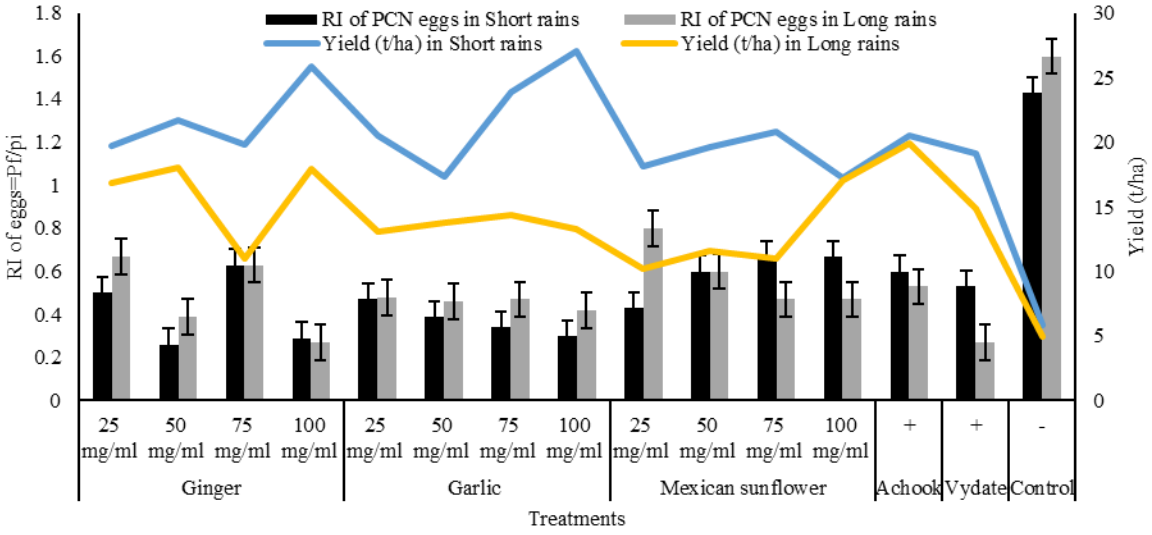

Figure 6.12. Effect of plant extracts on the reproduction rate of PCN and potato yield under field conditions

Effects of extracts on the PCN eggs and tuber yield

In all the treatments, PCN egg numbers declined (Figure 6.12). A decline in PCN egg numbers corresponded with an increase in potato yield. There was no consistent data on increase in extraction concentration on yield and PCN reproduction rate. Among the plant extracts, ginger extracts at 50 and 100 mg/ml had a maximum reduction in PCN eggs reproduction with RI ranging from 0.26 to 0.39 over the seasons followed by plants that received garlic at concentration of 50 and 100 mg/ml which showed RI of 0.3 and 0.46. Extracts of ginger at a concentration of 50 and 100 mg/ml and garlic at 100 mg/ml caused an increase in yield ranging from 17.99-27.09 t ha^{-1}. The maximum reproduction indices of RI = 1.43 and 1.6 of cyst were observed in the untreated plants during short rains and long rain, respectively and this led to low yield of 5.85 and 4.93 t ha^{-1}.

6.5.4 Phytochemical constituents of hexane extracts of ginger, garlic and Mexican sunflower

The qualitative and quantitative analysis of the ginger, garlic and Mexican sunflower hexane extracts revealed presence of alkaloids, steroids, phenols, terpenoids, glycosides and tannins (Table 6.9). The results revealed that hexane extracts of ginger had the highest percentage of alkaloids, saponins, flavonoids, phenols, terpenoids, glycosides and tannins among the plant extracts. Mexican sunflower had the least percentage of the phytochemicals present (Table 6.9).

Table 6.9. Qualitative and quantitative analysis of phytochemicals of hexane extracts from ginger, garlic and Mexican sunflower

Phytochemical compounds	Qualitative			Quantitative (%)		
	Ginger	Garlic	Mexican sunflower	Ginger (%)	Garlic (%)	Mexican sunflower (%)
Alkaloids	+	+	+	96.0	48.0	16.0
Saponins	+	+	+	26.4	3.9	1.6
Flavonoids	+	+	+	70.7	41.2	30.0
Phenols	+	+	+	81.9	59.6	25.4
Terpenoids	+	+	+	30.0	20.0	30.0
Glycosides	+	+	+	87.8	34.6	28.4
Tannins	+	+	+	64.1	62.4	57.8

+: Present

6.6 Discussion

The bio-assays results revealed that three of the 10 plant extracts; garlic, Mexican sunflower and ginger showed a higher potency than other extracts in reducing PCN egg hatch, loss of PCN egg viability and juvenile mortality. It was observed that after treatment of potato cyst nematode eggs and infective juveniles with garlic, Mexican sunflower and ginger extracts, the internal organs gradually disintegrated and became indistinct. This study is the first to report on effects of these plant extracts on PCN. Similar observations that were made on J2s and eggs of the root knot nematode *Meloidogyne javanica* on intestine damage after treatment with Camellia seed cake (Yang *et al.,* 2015). Furthermore, Khan *et al*. (2019) observed that the cytoplasmic membrane of *Meloidogyne incognita* was dissolved when exposed to plant extracts. In this study, it was observed that the dead J2s had four body shapes - straight, slightly bent, curved or sigmoid. The changes in body shape might be because of the toxic effects of phytochemicals that affect the nervous system (Taniwiryono *et al.,* 2009). This is attributed to substances such as alkaloids which are reported to affect the nervous system causing paralysis

and saponins which cause disintegration of internal organs and membrane alteration (Correia, 2014). Therefore, it can be assumed that the immobility and morphological change observed are related to the effect of the extracts assayed.

Contrary to Solanaceae crops being hosts of potato cyst nematodes enhancing egg hatch and maintaining egg viability, this study found that Sodom apple fruit extracts induced loss of PCN egg viability, reduced egg hatch and increased mortality of the infective juveniles under *in-vitro* conditions. This finding is probably because fruits and not the roots were used as the source of the extracts. In Solanaceae crops, it is the root exudates that induce hatching. It is possible that there were interactions between hatching factors and other chemicals such as solanine present in Sodom apple, which can cause both inhibition and stimulation of hatching factors depending on the solanine concentration. In support of this speculation, a high concentration of solanine in Sodom apple fruit extract was reported by Byrne *et al*. (1998) to inhibit hatching activity of the potato cyst nematode eggs in potato root leachate.

Plant species, plant part and type of solvent (water or alcoholic), and nematode species play important roles in achieving different nematicidal effects. In this study, plants extracts were obtained from natural sources using different solvents. The results demonstrated that water extracts of all the tested plant extracts showed the least nematicidal effect on loss of egg viability of potato cyst nematodes compared to methanol, ethyl acetate, hexane extracts. This suggests that bioactive compounds that are nematostatic in nature were not water soluble. Seenivasan (2019) made a similar observation where water extracts of all tested plant species extracts did not show any inhibitory effect on the banana nematode (*Radopholus similis*) compared to ethyl acetate, hexane and ethyl acetate which caused immobility on R. similis. According to Gupta *et al*. (2013) alcoholic extracts of *Datura stramonium* contain more chemical components than aqueous extracts. In this investigation, hexane extracts were found to have the strongest nematicidal activity against PCN eggs in comparison to ethyl acetate and methanol plant extracts which exhibited moderate activity. It is probable that hexane extracts were richer in the levels of bioactive compounds thus the superior performance of its extracts relative to the other extracts. This finding is supported by Siti *et al*. (2019) who found out that when hexane was used as a solvent, the extraction efficiency of bioactive compounds in *Vernonia amygdalina* leaf extracts such as phenolics isothiocyanates, glycosides, thiophenics, alkaloids, and fatty acids considered nematicidal in nature were enhanced. Differences in

performance of the plant extracts on potato cyst nematodes mortality of J2s, egg hatch inhibition and egg loss of viability were observed. This could be attributed to the differences in polarities of the four solvents used in this study, which have different solubility levels of phytochemicals leading to a wide variation in the level of bioactive compounds in the extracts used in this study (Seenivasan, 2019).

In the present study, it was also found that loss of egg viability of potato cyst nematodes increased with the exposure time to the plant extracts. Ginger, Mexican sunflower and garlic extracts exhibited the highest nematicidal activity of both eggs and juveniles of potato cyst nematodes at 72 hrs of the bioassay period. This finding agrees with a previous study, which found that plant extracts increased *M. incognita* juveniles mortality as exposure time increased (Abdalla *et al.,* 2008). This inconsistency of the juveniles' mortality rate with could be as a result of the larvicidal action value of the extracts which vary from one plant to the other. The solvents used in the present study are volatile and were allowed to evaporate during the extraction process. It is therefore, assumed they had non-contact nematicidal activity against PCN, so the extracts had only the bioactive compounds from the plants.

To compliment *in vitro* tests, *in-vivo* experiments (Section 6.3.5) were carried out to determine the effectiveness of hexane extracts of ginger, garlic and Mexican sunflower. These treatments significantly reduced the number of cysts and led to a low reproduction rate of PCN.in both field and screenhouse experiments when compared to the untreated control. For instance, garlic and ginger extracts at 100 mg/ml caused a significantly lower reproduction index of PCN eggs and cysts. These results compares with those of Youssef *et al*. (2016) who reported that garlic extracts had nematicidal effects against root knot nematodes on sugar beet. The findings of this study are in concurrence with Gupta and Sharma (1993) who indicated that garlic extracts when used as a drench caused 87-100 % J2 mortality of *M. incognita*, while Mexican sunflower extracts were reported to significantly reduce the number of eggs, number of juveniles and galls of *M. incognita* (Odeyemi and Adewale, 2011; Tsay *et al.,* 2004). Ginger extracts on the other hand were reported to suppress reproduction of *M. incognita* in tomato (Ibrahim, 2017)
According to Kouame (2021) suppression of nematode reproduction by garlic extracts is attributed to presence of pyruvic acid, allicin ammonia and sulfur compounds dimethyl-dipropyl, dimethyl-disulfide and diallyldisulphide. Allicin - a compound released by garlic was also found to be active against fungi and bacteria and inhibited reproduction of several plant

parasitic nematodes (Youssef *et al.,* 2016). It was evident in this study that ginger and garlic improved tuber yield significantly by 24.1-112.2 % over the two seasons of the study. The increase in yield in the potato plants that received the extracts could be attributed to a decrease in potato cyst nematodes infestation and low inoculum levels in the field. Studies by Hajihassani, *et al.* (2013) indicated that potato cyst nematode juveniles development within the root system reduce efficiency of uptake of water and nutrients into the roots consequently affecting tuber formation. The findings of this research are also in agreement with those of Lengai (2016) and Taye *et al*. (2012) who found that use of plant extracts reduced number of root knot nematode galls and egg masses on tomato roots leading to a remarkable increase in tomato growth and yield. In this study, ginger extract was the most effective in reducing the reproduction index of both the PCN cysts and eggs. It exhibited the highest nematicidal activity on cysts and eggs of PCN at LC20, L50 and L80. The high nematicidal action exhibited by ginger could be due to the high contents of the alkaloids, steroids, phenols, terpenoids, glycosides and tannins obtained from extracts. Agbenin *et al.* (2004) reported that extracts of ginger when applied as a drench in tomato fields suppressed the multiplication of *M. incognita* at concentrations above 10 %. Youssef *et al.* (2016) noticed that when ginger was applied as a soil drench at concentrations of 2.5, 5 and 10 %, a decrease in number of galls, egg masses and hatched juveniles in soil and roots of eggplant infected by *M. incognita.*

The qualitative phytochemical analysis performed in this study on hexane extracts of ginger, garlic and Mexican sunflower revealed presence of alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and phenols. Auger, *et al*. (2004); Gupta and Sharma (1993) indicated that garlic contains bioactive compound such as sulphuric acid compounds (allicin and diallylsulphuric), pyruvic and ammonia; Tona *et al.* (2000) observed that Mexican sunflower had saponins and alkaloids, whereas Ibrahim, (2017) recorded that ginger had alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and phenols all of which have been reported to confer resistance to nematodes. The exhibition of nematicidal effects on potato cyst nematode J2s and eggs by the three extracts could be linked to the presence of bioactive compounds. These extracts nematicidal potency could be attributed to the presence of the bio-active compounds. Previous research has shown that garlic extracts contain phytochemicals compounds which are allelopathic in nature and are toxic to nematodes (Nigh, 1985). According to Ibrahim (2017) ginger rhizome contains important phytochemicals such as tannin, terpenoids, flavonoids, saponins, glycosides phenols and alkaloid which have shown to

be effective nematicide. These findings are in agreement with those of Arora *et al.* (2012) who showed that ginger effectively reduced populations of leaf cutting beetle of mangoes resulting in high yield. Similarly, Kouame *et al.* (2021) reported that garlic produced phytochemicals that reduced root knot nematodes populations in tomato field. The quantitative phytochemical analysis performed in this study revealed that the three plant extracts showed different concentration of phytochemicals. This may have resulted in differences in reproduction indices of PCN. Similarly, Amaranth spinosus, Achyranthes aspera, Alternanthera pungens Kunth extracts were reported to show a varying degree of nematicidal effects on Meloidogyne incognita in vitro due to the active compounds present in each extracts (Ansari *et al.*, 2020).
Besides the direct nematicidal effects of these test extracts, there is a possibility that they might be taken up by host roots thus modifying host recognition by the nematode and they may also change the rhizosphere of the host crop thus protecting the roots from infective juveniles. Studies conducted by Mwamba (2016) illustrated that volatile organic compounds of Tagetes spp. influenced host seeking behavior by repelling of *Meloidogyne incognita* around the root rhizosphere and when taken systemically by the plant.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMEDATIONS

7.1 General discussion

The morphometric data in this study were inconclusive in differentiating *G. pallida* from *G. rostochiensis.* The perineal region and second stage juvenile morphometric data were inconsistent among the PCN samples collected from Nakuru, Nyandarua and Meru. For molecular identification, specific primers; ITS5/PITSp4 specific for *G. pallida* and ITS5/ PITSr3 specific for *G. rostochiensis* were used to discriminate the two species. Only *Globodera. rostochiensis* was detected in all the samples tested. Deliberate efforts must therefore, be made to prevent introduction of *G. pallida* in the areas used in this study to avoid challenges associated with managing it. *Globodera pallida* is considered more difficult to manage than *G. rostochiensis* because there is currently less resistance available in commercially-grown potato cultivars as compared to *G. rostochiensis* (CABI, 2020).

The PCN samples from Nyandarua, Nakuru and Meru counties displayed low genetic diversities confirmed by the fixation index, PCoA and phylogenetic analysis. One of the designed primer GRM2 used in this study was polymorphic and therefore can be used in determination of genetic diversity of *G. rostochiensis*. The polymorphic information content (PIC) values generated in this study were more than 0.25 and are comparable to those reported in other studies indicating that the four SSR markers were suitable for PCN genetic diversity evaluation. Three of the microsatelite markers (Gr 67, Gr79, Gr90) used in this study have been reported to show intermediate PIC (0.35 > PIC > 0.15) by Boucher *et al.* (2013). The phylogenetic tree formed two clades that had mixed samples from the three counties Nyandarua, Nakuru and Meru. This suggests that that the population of *G. rostochiensis* assessed in this study is likely to be composed a single lineage. The study revealed low genetic variation within the PCN samples collected from the three counties. This could be due to low gene flow in the populations studied as a result of limited mating and limited dispersal of the nematodes. According to Wang *et al.* (2017), only the second stage juveniles and males are mobile, resulting in a very low dispersal of PCN.

From the survey in chapter four, the results revealed variation in potato cyst nematodes population density across the three AEZs. For instance, higher cysts densities were observed in UH3 (289.2 cysts/300 g soil) and UH2 (203.7 cysts/300 g soil) AEZs which were cooler (11-

25ºC) and had higher rainfall (> 1000 mm) as opposed to a warm temperature (> 25ºC) and low rainfall (< 1000) mm in LH4 (63.1 cysts/300 g soil) AEZ. These findings were similar to those of Compton (2015) who found out that cyst population densities were different in regions and attributed this variability to difference in environmental conditions such as rainfall, temperature, soil properties and cropping practices. This finding is consistent with previous studies where a high percentage egg hatch of *G. rostochiensis* occurred at 10 to 25ºC (Kaczmarek *et al.,* 2014) and PCN was reported to reproduce significantly in cooler and high rainfall regions (Yusianto *et al.,* 2020).

Poor cropping practices and farmer's lack of knowledge on PCN and its management as observed in the survey might have contributed to the PCN spread and population density in all the AEZs. For instance, higher PCN densities were recovered in farms where continuous potato cropping was practiced compared to farms that practiced rotation. High PCN population densities were also observed in farms where informal seed potato was grown compared to farms where certified seed potato was used. Furthermore, crop rotation and the use of high quality seed have previously been identified as important aspects of PCN control (Mburu *et al.,* 2020). Additionally, the study area is famous of propagating variety Shangi, whereas over 80 % of farmers grew the variety. The status of Shangi variety as a good host for PCN has been previously reported (Mburu *et al.,* 2020). Continuous cultivation of the variety without adequate control measures is likely to exacerbate the spread of PCN, which will negatively affect production of the crop in the future. It may be prudent to accelerate the search for varieties with resistance to the PCN as a sustainable approach to managing the pest (Djebroune *et al.,* 2021). The high PCN density and its prevalence in the study areas can be explained by the fact that farmers are not using any control measures to manage PCN. These findings suggest that in order for PCN to be effectively controlled, farmers must have access to suitable rotations and disease free seed as part of an integrated pest management strategy for the pest.

The current demonstrated that the population density of PCN varied significantly depending on soil texture. According to the study, high PCN population densities were identified in soils with sand ≥ 50 %, silt ≥ 20 % and clay ≤ 28-37 %. Soils having ≥ 50 % sand are well aerated and had high moisture content. Soil texture affects nematode population density and distribution by restricting their movements towards the host (Dana, 2004). This finding is consistent with previous research that found that root knot nematode damage to be greater in

sandy soils than in clay soils (Barker and Weeks, 1991; Shane and Barker, 1986). The sandy clay soils and sandy clay loam soil had the lowest mean number of cysts. According to Dana (2004), clay soils are termed as nematode suppressive soils because of the restricted movement resulting to less root invasion. Prot and Van Gundy (1981) demonstrated that soil texture influenced nematode spread and density, with *Meloidogyne incoginita* mobility inhibited in clay soil, reducing their population.

This study showed that the magnitude of yield losses caused by PCN was dependent on susceptibility of the variety, the nematode initial population density and effectiveness of the PCN control product. The PCN resistant cultivar had the lowest yield loss. The ability of resistant varieties to suppress PCN could be attributed to a gene that confers resistance against PCN. According to Mburu, *et al*. (2020), cultivars with the H1 gene exhibit resistance through interference with multiplication of resistant to PCN. Usually the roots of PCN resistant cultivars are invaded by second stage juveniles but their development is restricted due to necrotic degeneration of synctial feeding cells (Lamondia and Brodie, 1986). Previous studies have shown that PCN resistant cultivars can significantly reduce PCN population thus resulting to less yield losses. For instance, Lamondia and Brodie (1986) demonstrated that *G. rostochiensis* densities were reduced up to 95 % each season that a resistant variety was cultivated. Conversely, high PCN reproduction which resulted into high yield loss was recorded in a PCN susceptible variety. This is attributed to lack of a gene which codes for PCN resistance Mburu *et al.* (2020). According to Faggian *et al.* (2012), *G. rostochiensis* susceptible varieties can increase the population densities of cysts to 2-35 times.

A negative correlation between the PCN multiplication (RI) and initial population density (Pi) was observed in this study. For example, a high PCN reproduction was observed in low PCN infestated and vice versa. This may be due to competition for nutrients and space in roots, whereby low inoculum in the field leads to less competition for finding host, nutrients and feeding sites, resulting in a high PCN invasion in the roots. Additionally, the female J2s are able to mature in relatively low PCN infested farms due to less competation nutritionally than in high PCN infested farms (Trudgill, 1986). This is in agreement with Kaczmarek (2022) who observed a decrease in *G. rostochiensis* muiltiplication when variety Vales Everest was grown in heavily PCN infested field.

In this study, the PCN control products were effective in reducing PCN multiplication and yield losses. PCN control products affect multiple nematode life stages restricting feeding, movement and hatching and causing mortality. Once paralysed the nematodes take on a needle-like appearance and are unable to enter the roots to feed, dying soon after (EPPO, 2017). However, the costs some of the effective nematicides such as oxamyl were quite high and may be out of reach for small scale farmers in the county.

The potency of extracts of Mexican sunflower, garlic, ginger, Mexican marigold, spring onion, sodom apple, eucalyptus leaves, eucalyptus bark, green tea leaves and onion bulb in management of PCN under *in-vitro* conditions was studied (Chapter 6.0). The results showed that the ten plant extracts inhibited egg hatch, caused egg viability loss and increasing J2s mortality. Under *in-vitro* conditions, hexane extracts of garlic, ginger, Mexican sunflower had the greatest nematicidal effects on egg and juvenile. Previous studies have shown nematicidal activity of plant extracts belong to *Meliaceae*, *Asteraceae*, *Myrtaceae*, *Amaryllidaceae*, *Theaceae*, *Zingiberaceae*, and *Alliaceae* families (Boulogne *et al.*, 2012; Okwute, 2012). Botanical extracts have been used in disease and pest control probably because they contain phytochemicals which are pesticidal. In the current study, phytochemicals analysis of hexane extracts of ginger, garlic and Mexican sunflower revealed presence of alkaloids, tannin, phenols, glucosinolates, saponins, flavonoids, and terpenoids. These phytochemicals have been shown to have nematicidal properties (Ansari *et al.*, 2020). The results showed that these compounds varied quantitatively and qualitatively in their chemical composition in each plant. These variations in phytochemicals may be due to species / variety or age of the plant (González-Mas *et al.*, 2011, Agbenin *et al*. 2004).

The greenhouse and field results showed that hexane extracts of ginger, garlic and Mexican sunflower significantly caused loss of egg viability and J2s mortality. Hexane extracts of ginger at 100g/ml exhibited the greatest loss of egg viability and juvenile mortality both in the greenhouse and field. Ginger exhibited a highest nematicidal activity on cysts and eggs of PCN at LC_{20}, L_{50} and L_{80}. The nematicidal activity revealed by ginger extracts could be as a result of high contents of the alkaloids, steroids, phenols, terpenoids, glycosides and tannins. The findings are consistent with the findings of Agbenin *et al*. (2004) who reported that extracts of ginger when applied as a drench in tomato field suppressed the multiplication of *M. incognita* and attributed their nematicidal effects to high contents of oxygenated compound.

7.2 Conclusions

This study showed that *Globodera rostochiensis* was the only PCN species detected in the three counties. Additionally, we found that the potato cyst nematode (*G. rostochensis* species) had low levels of genetic diversity. The low genetic variation found within the PCN is useful information that can be used in breeding of resistant *G. rostochiensis* varieties and developing appropriate nematode management strategies.

According to the findings of this study, site specific factors such as soil characteristics, farming and cropping practices interact to determine the prevalence and population density of PCN. In addition, the findings re-affirm the need for an integrated approach incorporating crop rotation, planting of disease-free tubers, resistant varieties and field sanitation as well as enhancing farmers' knowledge on management of the pest to reduce the spread and build-up of PCN.
The relative yield losses attributed to PCN infestation varied depending on the initial PCN infestation, potato cultivar and could be as high as 80.5%. It has been demonstrated that PCN can be effectively managed using resistant potato varieties and PCN control products. Among the nematicides, oxamyl was the most effective nematicide in reducing PCN numbers and yield losses however, it was withdrawn from the market in 2022 due to the risks it poses to humans and environment.

It was established that hexane extracts of ginger and garlic at 100 mg/l were effective in the management of PCN and resulted into high yields. These extracts were effective in inhibiting PCN egg hatch, loss of PCN egg viability, causing juvenile mortality and reduction of cyst counts in both *in-vitro* and *in-vivo* experiments. The active ingredients of hexane extracts of ginger, garlic and Mexican sunflower were alkaloids, flavonoids, saponins phenols, terpenoids, glycosides and tannins and varied in quantitative composition. Ginger extracts had the highest concentration of these phytochemicals.

7.3 Recommendations

1. Farmers should be encouraged to adopt integrated pest management practices incorporating use of certified seeds, crop rotation, resistant varieties, field sanitation and appropriate nematicides to prevent further spread and build-up of PCN population thereby reducing yield losses caused by PCN
2. The absence of *G. pallida* which is more destructive to potato crops calls for deliberate efforts to exclude it from the country as well as the potato production sites
3. Application of hexane extracts from ginger and garlic at 100 mg/l should be encouraged since it will help in reducing cysts population densities
4. Studies on genetic diversity should be extended to other PCN populations present in other potato growing counties in the country.
5. Regular surveys should be conducted to monitor PCN species present, their prevalence and population densities in the field to enable farmers deploy the appropriate integrated management practices
6. Other commonly available plants with potential as botanical pesticides should be evaluated to increase chances of identifying potent extracts that can be developed into commercial products for managing PCN.

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APPENDICES

Appendix 1. Tool for data collection on factors that contribute to spread and build-up of potato cyst nematode population in selected agro-ecological zones in Nyandarua county

1. Name of the county……. Sub-county……... Ward……...

2. a) Name of farmer ……... b) Gender 1. Male 2. Female

Date……

3. Sample collected coding number……

4. Agro ecological zone 1. UH1 2.UH2, 3. LH (Phone activated GPS) 1. Longitude 2. Latitude 3. Altitude

5. a) What is the total land size (acre) …….

b) Land tenure 1) Owned, 2) Leased

6. Size of land devoted to potato (acre) per season 1. ≤0.5, 2. 0.6- 1.0, 3. 1.1- 1.5, 4. 1.6-2.0, 5. 2.1-2.5, 6. ≥ 2.6

7. a) Do you practice crop rotation 0. No, 1. Yes

b) If yes, which crops are rotated with potato 1. Peas, 2. Carrots, 3. Cabbages, 4. Maize, 5. Kales, 6. Beans 7. Snow peas 8. Any other (specify)

8. a) Over what duration (in years) has the land been under potato? 1. One, 2. Two 3. Three 4. Four, 5. Five, 6. Six, 7. Seven 8. Eight 9. Nine, 10. Ten 11. None

b) Number of potato crops grown in the same field per year 1. One, 2. Two, 3. Three, 4. Four

9. a) Do you grow potato as 1. Intercrop, 2. Monocrop, 3. Relay crop

b. If intercrop, record the crop grown together with potato 1. Peas, 2. Carrots, 3. Cabbages, 4. Maize, 5. Kales, 6. Beans, 7. Snow peas, 8. Any other (specify)

10. What is the cropping history of the potato land?

Cropping history	**Crop (codes)**
Last season	
previous season	
2nd previous season	
3rd previous season	

Codes; 1. Peas, 2. Carrots 3. Cabbages, 4. Maize, 5. Kales, 6. Beans, 7. Snow peas 8. Any other (specify) 9. None

11. In the table below, give farm operations, done by who, source of tools and the treatment used to treat the tools used (Fill the table below as appropriate)

Farm operations	11.1Done by Who (*codes*)	11.2. Source of tools (*codes*)	11.3.Treatment used to disinfect the tool(*codes*)
Ploughing			
Harrowing			
Planting			
Weeding			
Harvesting			

	Done by who	Source of tools	Treatment used
	1.Hired worker	1. Own tractor	1. Jik
	2.Owner	2. Workers tools	2. wáter
		3. Own hand tools	3. Kerol
		4. Hired tractor	4. Dettol
		5. Others	5.None

12. In table 1 below give variety grown, initial source of seed, when was initial seed acquired, when was additional seed acquired and from where

Table 1. Variety grown, source of seed grown and when was intial and additional seed acquired

12.1Variety grown	12.2 Initial source of seed	12.3 When was seed acquired (Years)	12.4 When was additional seed acquired (Years)	12.5 Ad Seed fron
Codes				
Variety grown	Initial/additional source of seed / from where	When was initial/ additional seed acquired (year)		
1.Shangi	1. Own seed	1. one		
2. Dutch	2. Market	2. Two		
3.Sherekea	3.Neighbours	3. Three		
4. K. Karibu	4.Certifiedseed growers	4. Four		

5. K. Mpya	5.Other sources Specify	5. Five		
6.Tigoni		6. Six		
7.Others(specify)		7. Seven		
		8. Eight		

13. What form of seed do you plant? 1. Whole tuber, 2. Sliced tubers, 3. Rooted apical cuttings

14. What is the frequency of seed renewal (Years)? 0. Never, 1. 1-3, 2. 4-6, 3. 7-9, 4. 10-11,

15. Fertilizer type used (Table 2.)

Table 2 Type of fertilizer used and when is it applied

Type of fertilizer	15.1 At planting	15.2 During top dressing
inorganic		
organic		
Others (Specify)		
None		

Codes; Inorganic fertilizer 1. DAP, 2. NPK, 3. CAN 4. UREA 5. Wuxal 6. Others (specify) Organic fertilizer; 1. Manure, 2. Biovartilizer 3. Others (specify)

16. a.) What are the diseases and pests affecting your potato crop?

1. Bacterial wilt , 2. Late blight, 3. Viruses, 4. Others (Specify)

b). What control measures do you practice?

1. Crop rotation, 2. Use of pesticide, 3. Rogueing, 4. Use of fungicides, 5. Use of border crops, 6. None, 7. Others (specify)

17. a) Do you know the pest called Potato Cyst Nematode (PCN)? 0. No 1. Yes

b) If yes, can you please describe the symptoms?

1. Yellowing, 2. Stunted growth, 3. Dwarfness, 4. Wilting, 5. Drynesss, 6. None

c) Is PCN a problem in your farm?. 0. No, 1. Yes 3. Don't know

18. a) Potato yield in an acre in 50kg bags before PCN attack (bags/acre) 1. 1-10, 2. 11-20 3. 21-30, 4. 31-40, 5. 41-50, 6. 51-60, 7. 61-70, 8. 71-80

b) Potato yield in an acre in 50kg bags after PCN attack (bags/acre). 1. 1-10, 2. 11-20 3. 21-30, 4. 31-40, 5. 41-50, 6. 51-60, 7. 61-70, 8. 71-80

19. What is the yield loss associated with damage from PCN

a). Farmers opinion (50 kg bags/acre) …….

b). Estimation (50 kg bag) based on empty patches and stunting_____

20. a. What measures have you taken to respond to PCN attack 0. None 1. Uprooting 2. Crop rotation 3. Nematicide application 4. Use of trap crop

b. If uprooting, how do you dispose uprooted materials……...

c. If Pesticide application which nematicide 1. Vydate 2. Fluopyram 3. Biological nematicide 4. Botanical nematicide 5. Other (please specify)

21. Do you uproot the potato volunteer crops in your farm? 0) No, 1.Yes

22. What do you do with left over tubers after harvesting?

1. Nothing, 2. Collect and remove, 3. Collect and remove and put in a condemnation pit

Enumerator observations/ measurements

23. What are the diseases observed on the potato crop (enumerator to scout) 1. Bacterial wilt, 2. Viruses, 3. Rhizoctonia, 4. Blight, 5. PCN, 6. Any other (specify)……...

24. The symptoms that can be attributed to PCN in the farmer's field 1) Yellowing 2) Dwarfness, 3). Wilting, 4). Drynesss, 5) None

Spatial pattern of PCN damaged plants (above ground**)**

1). Uniform and restricted

2). Widespread and Uniform

3). Widespread and Scattered

4). Restricted and scattered

25. Prevalence of PCN cysts on randomly uprooted potato plants (Use the scale in table 3. below)

Table 3. Prevalence of PCN on randomly uprooted potato plants

Prevalence of PCN cyst on 3 uprooted plants	Score (codes)
1. plant one	
2. Plant Two	
3. Plant three	

Table 4. Scores of cyst count

Cyst count	Score (Codes)
1	9
2-3	8

4-5	7
6-10	6
11-15	5
16-25	4
26-50	3
51-100	2
>100	1

Score 9 indicate the least number of counts Source EPPO (2006)

26. Current PCN severity (to do scouting during survey and count) (%) during interview in three independent plots. Refer to Table 1. For scoring

26.1a. Plot 1 Severity score ……. %

26.1b. Stage of growth 1. Pre flowering, 2. Flowering, 3. Post flowering

26.2a. Plot 2 Severity score……. %

26.2b. Stage of growth 1. Pre flowering, 2. Flowering 3. Post flowering

26.3 a. Plot 3 Severity Score……. %

26.3b. Stage of crop growth 1. Pre flowering 2. Flowering 3. Post Flowering

27. a) Number of plants infested by PCN ……

b) What proportion of field in percentage is infested by PCN percentage……...%

28. Asses the PCN numbers from other solanaceous plants/weeds in the farmer's field (use Table 5. above).

Table 5. PCN count on roots of other solanaceous plants

Solanaceous plants/weeds	PCN numbers per plant
1. Night shade (vilosum).	
2. Night shade (scubrum)	
3. Egg plant	
4. Tomatoes	
5. Thorn apple	
6. None	
7. others (specify)	

29a) Are there PCN cysts observed on volunteer potato plants 0. NO, 1.YES

b.) No. of volunteer's potatoes in 1/8 of the fallow field 1. ≤ 50, 2. 100 3. 150 4. 200 5. ≥250 30. a) Occurrence of cysts on crops rotated or intercropped with potato 0. NO 1. YES

b) What rotational crops had recurrent PCN infestation 1. Peas… 2. Carrots…. 3. Cabbages, 4. Maize, 5. Kales, 6. Beans 7. Snow peas, 8. None, 9. Any other (Specify)

31. What abiotic (non-disease/pest related) factors that appear to affect the crop

1. Nutritional, 2. Water stress, 3. Flooding, 4 Poor drainage, 5. Shading effect from nearby trees/fruit trees 6. Others (specify)

32. What is soil type in the farmer's field? 1. Clay, 2. Clay silt loam, 3. Loam

33. What is soil texture in the farmer's field? 1) Clay, 2. Sand, 3. silt

34. Common weeds in the potato plots

1. Thorn apple, 2. Macdonald eye, 3. Black night Shade, 4. Chinese lantern, 5. Saw thistle

35. Is there potential for soil run off in the farmer's field 0. No, 1. YES

…….**Thank the Farmer and End Interview**