MANAGEMENT OF POTATO CYST (*Globodera rostochiensis* W.) NEMATODE USING HOST PLANT RESISTANCE, CHICKEN MANURE AND *Datura stramonium* L. EXTRACTS IN NAKURU COUNTY, KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Plant Pathology of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

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Recommendation

This thesis has been submitted with our approval as university supervisors.

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DEDICATION

To my dear husband Peter Macharia, children Blessing Muthoni, Bresnic Ithagu and Briana Wambui, parents Moses Kamau and Mary Wambui.

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ABSTRACT

Irish potato (*Solanum tuberosum* L.) is the second most important food crop in Kenya after maize and a source of employment and income for many families. In 2015, potato cyst nematode *(Globodera rostochiensis)* was first reported in Kenya. This nematode is a quarantine pest in many countries because of its adverse effects on potato production. The current study was carried out to manage the nematode using host plant resistance, plant extracts of *Datura stramonium* and chicken manure. Cysts of *G. rostochiensis* were extracted from infested soil collected from Molo and Njoro sub Counties. Six commonly grown potato varieties ie Shangi, Kenya Karibu, Sherekea, Tigoni, Kenya Mpya and Dutch Robjin were screened for resistance against the nematode using pot experiments. Each pot with a tuber was inoculated with 25 cysts. Each variety was replicated 4 times and arranged in a Completely Randomised Design (CRD) on raised benches. After 10 weeks the cysts from each pot were counted and compared to the susceptible standard variety (Desiree). All the tested varieties were susceptible with a relative susceptibility score of either 2 or 3. However there was a significant difference in cyst population among the test varieties ($F(5, 18) = 6.138$; $P = 0.002$). The effects of aqueous extracts of leaves, roots and seeds of *D. stramonium* on mortality of second stage juveniles (J2s) of *G. rostochiensis* was tested *in vitro* in Egerton University, Department of Biological Sciences laboratory. Twenty freshly hatched J2s were exposed to 5 ml of each extract at dilutions of 100, 50, 25 and 12.5% while distilled water was used as a control. The 100 and 50% dilutions were the most effective, achieving 100% mortality within 24 h of exposure. The root extract was more effective as compared to the seed and leaf extracts after 24 and 48 h of exposure, however there was no significant difference among the three extracts after 72 h of exposure. Chicken manure was mixed with sterilized soil at a rate of 1:2 w/w manure to soil and used to plant the susceptible standard variety, Desiree. Unamended soil was included as a control. The population of *G. rostochiensis* in the amended soil was significantly low when compared to the unamended soil at ($t(6) = 4.138$, $p < 0.006$). The performance of the test varieties was subjected to one way ANOVA using SPSS program (version 24). The effect of the aqueous extracts was determined by a two way ANOVA using SPSS program (version 24) while the amended and unamended soils were compared using two tailed t- test. This study established that extracts of *D. stramonium* had nematicidal effects on the J2s. the extracts can be explored for formulation of a bionematicide. The chicken manure was effective in reduction of *G. rostochiensis* cysts and therefore can be recommended for integrated pest management on the farm.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Agricultural Development Corporation
AHDB	Agriculture and Horticulture Development Board
APPPC	Asia and Pacific Plant Protection Commission
ASDS	Agriculture Sector Development Strategy
CABI	Centre for Agriculture and Biosciences International
CPPC	Carribean Plant Protection Commission
DAP	Diammonium Phosphate
EPPO `	European and Mediterranean Plant Protection Organisation
EU	European Union
FAO	Food and Agriculture Organisation
FERA	Food and Environment Research Agency
GOK	Government of Kenya
IAPSC	Inter- African Phytosanitary Council
J2S	Second Stage Juveniles
KALRO	Kenya Agricultural and Livestock Research Organisation
KEPHIS	Kenya Plant Health Inspectorate service
LSD	Least Significant Difference
MAFF	Ministry of Agriculture, Fisheries and Food
MoALF	Ministry of Agriculture Livestock and Fisheries
NAPPO	The North American Plant Protection Organisation
NCIDP	Nakuru County Integrated Development Plan
NIVAP	Netherlands Potato Consultative Foundation
NPCK	National Potato Council of Kenya
PCN	Potato Cyst Nematode
PRD	Potato Root Diffusate
RF	Reproductive Factor
RS	Relative Susceptibility
SRS	Statni Rostlinolekarska Sprava (State Phytosanitary Administration)
UK	United Kingdom
USDA-ARS	United States Department of Agriculture–Agriculture Research Service

CHAPTER ONE
INTRODUCTION

1.1 Background information

The nematode, *Globodera rostochiensis* W. is a worldwide pest of temperate regions of the world (CABI, 2021). It is considered to have originated from Andes region of South America, from where it spread to other potato growing regions. There are three main species of *Globodera* that attack plants i.e. *G. rostochiensis*, *G. ellingtonae* and *G. pallida* (Whitworth *et al.,* 2018) and they are collectively referred to as Potato Cyst Nematodes (PCN). These nematodes attack about 150 known plant spp. in the genus *Solanum*. A few species of *Datura, Hyoscyamus, Lycopersicon, Physalis*, *Physoclaina*, *Salpiglossis* and *Saracha* have shown that they can host PCN where they occur and also allow their reproduction (Sullivan *et al.*, 2007). Studies have shown that for every 20 eggs per gram of soil, there can be up to 1 ton per acre yield loss due to poor growth and reduced tuber sizes. If uncontrolled, this nematode can cause up to 80% loss in yield (Dandurand *et al.*, 2016). In Chile, yield losses of 20, 50 and 90%, were obtained in population densities of 9, 28 and 128 eggs/g of soil respectively in microplots planted in summer (Greco & Moreno, 1992). This indicates that the higher the PCN population, the higher the yield losses in a potato crop. *Globodera rostochiensis* is a quarantine pest for European and Mediterranean Plant Protection Organisation (EPPO), Asia and Pacific Plant Protection Commission (APPPC), The North American Plant Protection Organisation (NAPPO) countries and equally significant in the Carribean Plant Protection Commission (CPPC) and Inter- African Phytosanitary Council (IAPSC) regions (FAO, 2021). This means the presence of PCN in a potato growing area prevents the export of potatoes to international markets due to the restrictions imposed by many countries against this pest.

The *G. rostochiensis* is also associated with other soil pathogens causing complexes that lead to further crop loss. In a field study carried out on *G. rostochiensis,* juvenile population increased with incidence of stolons infected by *R. solani* (Back *et al*., 2002). This leads to further crop loss which has direct effect on the profitability of the grown crop. The females also cause extensive damage on the roots which impairs the absorption of water in the plants leading to wilting and stunting (Dutta *et al.,* 2019). The *G. rostochiensis* and *Verticillium dahliae* complexes are highly destructive since they result to early dying of potato plants (Evans, 1987) thus it results to total crop failure where the complex occurs.

In Kenya, *G. rostochiensis* was first reported in April 2015 in Nyandarua County (Mwangi *et al.,* 2015) after which *G. pallida* was reported in 2018 (Mburu *et al.,*2018). Similar reports have been made from other east African countries such as Rwanda (Niragire *et al.,* 2019) and Uganda (Cortada *et al.,* 2020). This makes the challenge to be more of regional as opposed to being national. Considering the gross effects of the pest in other potato growing areas, the reports called for need to identify ways of managing the pest and implementing proper measures to control further spread of the nematode. Being a relatively new problem, there is no local potato variety is known to be resistant to the nematode but a few imported seeds are registered as resistant (NPCK, 2017). This study therefore, was aimed at identifying potato varieties currently grown in Njoro and Molo sub- counties that are resistant to *G. rostochiensis.* The use of chicken manure and plant extracts of *D. stramonium* to manage the nematode was also assessed.

1.2 Statement of the problem

In 2015, *G. rostochiensis* was reported in Kenya. The nematode is a registered quarantine pest by many countries, which means that export of products from infested area face major restrictions. Once established in an area, the eggs can remain viable in the soil for more than 30 years. The nematode is difficult to eliminate from an area which leads to high losses in potato yield and income in subsequent years. This has led to some countries restricting the production of potatoes in the infested areas for up to 40 years. Currently, there is no readily available method in Kenya to manage the nematode. The potato varieties grown by the farmers have also not been screened for resistance against the nematode. The nematode cases reported by isolated farmers presently, can cause substantial potato yield losses from the nematodes if they become widespread. This is due to negative effects of the nematode on the potato such as poor growth rate, few and small sized tubers and damage of the root system which impairs the absorption of nutrients and water. Therefore, there was an urgent need to assess how the nematodes can be managed before they cripple potato industry in Kenya.

1.3 Objectives

1.3.1 General objective

To improve potato production through the management of potato cyst nematode disease.

1.3.2 Specific objectives

i) To screen commonly grown potato varieties for resistance against *G. rostochiensis* in Njoro and Molo sub- counties.

ii) To determine the effect of chicken manure on the population of *G. rostochiensis*.

iii) To determine the effect of aqueous extracts of *D. stramonium* on the mortality of *G. rostochiensis* juveniles.

1.3.3 Hypotheses

i) There are no commonly grown potato varieties in Njoro and Molo sub-counties that are resistant to *G. rostochiensis*.

ii) There is no significant effect in application of chicken manure on the population of *G. rostochiensis*

iii) There is no significant effect in application of *D. stramonium* extracts on the mortality of *G. rostochiensis* juveniles

1.4 Justification

Potato is the second most important food crop in Kenya after maize and is an important source of food, employment and income in developing countries including Kenya. Potatoes in Kenya contribute Kshs 50 billion annually after maize which contributes Kshs 120 billion per year to the economy. The report of a serious potato pest *G. rostochiensis* in the country is a threat to potato production. The nematode is known to cause up to 80% yield loss in potato growing areas where it is established and can persist in the soil for a long time. This would translate to a huge loss to the country's and the farmers' income. The current study was aimed at screening six popularly grown potato varieties for resistance against *G. rostochiensis,* as a way of managing the nematode spread to other areas while maintaining high yields in potatoes. Also effect of application of chicken manure and plant extracts of *D. stramonium* was assessed in the management of the nematode. The above three methods are environmentally friendly. Management of *G. rostochiensis* in the infested areas will help to guard the country against its spread to the unaffected areas. This will be helpful especially where important vegetables belonging to genus *Solanum* such as egg plants and tomatoes are grown. The study is in line with the Sustainable Development Goals (SDGs) number 2, 8, 12 and 15. It will also contribute towards attaining the Vision 2030, as strategized in the economic pillar sector 2 and social pillar sector 7. This will increase potato production in the country, leading to improved living standards and increased national food security.

CHAPTER TWO
LITERATURE REVIEW

2.1 Potato crop review

Potato (*Solanum tuberosum* L.), belongs to the solanaceae family of flowering plants. It is grown for stem tubers which are eaten after cooking or processed into crisps, chips, flour, juices, starch and other products (Laititi, 2014). It is known to have a high energy component. In the world, potato is the third most important food crop after rice and wheat in terms of human consumption and the fourth in terms of production after rice, wheat and maize (FAOSTAT, 2018). Potato was introduced in Kenya in 1880s and since then it has been grown in the country. Over the past 40 years or so, it has gained popularity among many communities in the country, becoming the second most important food crop after maize (MOA, 2015). The popularity is mainly due to the increased urbanization, high population growth and general growth in the fast of the fast food industry in the country. Irish potatoes are mainly grown by small scale farmers in the cool highland areas. In Kenya it is considered as a high quality and an important food item which is a staple food and also a source of income (Muthoni *et al.*, 2013). It is an easy to produce crop and this makes it to be easily incoporated in peri-urban agriculture which provides jobs and food security to some 800 million people globally (Hoffler & Ochieng, 2009). The nutrient components of potato~~es~~ include carbohydrates in form of starch (which accounts for 60-80% of the dry matter), Vitamin C (when potato is eaten with its skin), Vitamins B_1, B_3 and B_6 and minerals such as Potassium, Phosphorous and Magnesium. Also, folate, pantethetic acid, riboflavin and antioxidants are present in small amounts (Beals, 2019). The common potato varieties grown in Kenya include Shangi, Tigoni, Kenya Karibu Asante, Komesha, Nyayo and Thimathuti (Muthoni *et al*., 2014). In Nakuru County, the common potato varieties that are grown include Shangi, Kenya Mpya, Kenya Karibu, Sherekea,Nyayo, Rudolf, Jerry and Curruso among others (MoALF, 2016).

2.1.1 Climatic conditions for potato growth

For tuber initiation, a night temperature of 15^{o} C is required and a soil temperature of 15-18^{o} C while for tuber growth, temperatures below 10 and above 30^{o} C adversely effects the tuber development (Muthoni *et al.*, 2013). In Kenya, potatoes are grown in the high attitude areas of 1200-3000 metres above the sea level which receive an average rainfall of 1000- 1800 mm. These areas include the slopes of Mt. Kenya, the Mau escarpments, the Nandi escarpments, Cherangany Hills and the slopes of Mt. Elgon. They are also grown in small quantities in Kericho, Kisii and the areas of Taita Hills (Kirumba *et al.,* 2004). The varieties grown take 3-

4 months to mature hence the farmer can complete three planting seasons per year. Because of this short duration, some farmers prefer growing potatoes over maize which takes about 10 months to mature (Kiiya *et al.,* 2006).

2.1.2 Economic importance of potatoes

Kenya being a developing country, potato is an important source of food, employment and income (Food and Agriculture Organisation of the United Nations (FAO), 2008). Presence and spreading of *G. rostochiensis* in the potato growing areas can have adverse economic impacts. Kenya produces about 2,192,885 tons of potatoes per year out of the World's 376,454,524 tons (FAOSTAT, 2018). Egypt was the leading potato producer in Africa in 2016 with a total yield of 5029020 MT per year. It was followed by Algeria, South Africa, Morocco and Kenya was position five with a yield of 1335880 MT per year (FAOSTAT, 2018). Potatoes in Kenya contribute Kshs 50 billion annually while maize contributes Kshs 120 billion per year (NPCK, 2015). Between 2012 and 2016, Kenya recorded a decline in potato production from 2915070 MT to 1335880 MT regardless of an increase in the area of production.

Potato is grown by about 800,000 small holder farmers in the country. The farmers usually allocate more than 25% of their farms to potatoes on average when compared to other farming activities integrated in the farms (Muthoni *et al.*, 2014). Land allocation however is also determined by farm sizes, because small holders allocate less land as compared to large farm holders. When working with potato producers in Mauche (Kenya), Taiy *et al.* (2016), noted that small holders (1-5 acres) allocated 1-1.5 acres to potatoes which agrees with the 25% allocation. According to GOK, 2010, the overall goal of Kenya is to achieve an average growth rate of 7% per year of agricultural productivity. With *G. rostochiensis* invasion, this goal will be difficult to achieve in potato production. Due to the effects of *G. rostochiensis,* some countries have imposed bans on the infested areas leading to the collapsing of the area's economy. For example in Norway, infestation by PCN, resulted to 40 years ban on growing potatoes in the infested area (Holgado & Magnusson, 2012). Repeated potato cultivation on infested land can lead to crop loss of up to 80% due to buildup of nematode population (Abd-Elgawad, 2020). Since most growers in Kenyan are smallholders, potato production is continuous throughout the year (Taiy *et al.*, 2016) and this would make the multiplication of the nematode faster. The Horticultural Crops Directorate (2016) reported that there was a marginal reduction in potato yield per unit area in Kenya from 12.86 ton/ha in 2015 to 12.76 ton/ha in 2016. This has a negative economic implication on the country's' economy since

potatoes contributes 41.2% of total value of vegetables in the country. Nakuru County produced a total of 403, 080 MT in 2016, which was 2nd highest producer in the country contributing to 18% of all potatoes produced (Horticultural Crops Directorate, 2016).

2.2 Constraints in potato production

The potato crop production in Kenya is faced by various constraints which include poor quality of seed potato, pest and diseases, low soil fertility, high input prices and low market prices (Muthoni *et al.*, 2013). Some of the constraints are discussed below.

2.2.1 Poor seed potato quality

Poor seed potato quality has been reported as one of the main contributing factors to low production in potatoes in sub- Sahara Africa (Fuglie, 2007). The quality of the potato seed used by the farmer is a major determining factor to the quantity and quality of the yield, which later translates to the amount of income and food received. Considering that most of potato growers operate on small scale, the have a tendency of using the harvested potatoes saved from previous crop as seeds or obtain the seeds from uncertified sources (Hudu *et al.,* 2018). This leads to accumulation of seed- borne diseases over time and eventual decline in yield and quality of the crop (Gildemacher *et al.,* 2007).

The seed quality is determined by two factors, the biological attributes and the physical characteristics of the seed (Wang, 2008). The biological aspects include the variety of the seed, age of the seed tubers, level of pest and disease infection and general degeneration. The seed potato variety is important as far as the yield is concerned. Different potato varieties show different levels of yields. For example in Kenya, some of the high yielding varieties include Shangi, Dutch Robjin, Asante, Desiree, Sherekea, Tigoni and Unica which can produce up to 30t/ ha (NCPK, 2017). The physical factors are the seed uniformity and the size. Medium sized seed potatoes give a better output as compared to large or small ones. The small seeds have little food reserve for plant development. Some of the recommended solutions to this problem is the use of true potato seeds and creating awareness to the farmers on where to get the certified seed potatoes (FAO, 2013). The farmers should also be educated on the importance of timeliness of the various operations so as to improve on the total output.

2.2.2 Poor soil health

Soil health refers to the general characteristic of the soil in regard to amounts of nutrients available to plants, soil pH, soil - borne pests and diseases and other factors that influence the sustainability of the soil to plant growth over a long period of time (Norris & Congreves, 2018). Irish potatoes grow well in loamy and sandy loam soils that are well drained and aerated. The organic matter content of the soli should be high with a pH range of between 5 and 6.5. The depth of the soli has a direct effect on the tuber development thus translating to better yields (Gildemancher *et al.,* 2009). Other than the soil properties, the spacing of the seed potatoes was reported to be another factor contributing to the overall potato yield. In a study carried out in some potato growing areas in Kenya 66% of the land have nutrient content below the critical levels. The study further showed that most of the areas had a low tissue nutrient concentrates for nitrogen, phosphorus, potassium and sulphur (Mugo *et al.,* 2020). Low soil fertility limits the output in potatoes directly. In Kenya, some potato growing areas are reported to have low soil fertility and also low pH which makes the nutrients to be imbalanced. Since the main fertilizer used for planting potatoes is Di- ammonium phosphate (DAP), the pH in most regions tend to be low (Muthoni, 2016). The farmers should be educated on the appropriate amount and type of fertilizer to use so as to improve the potato yield. This is expected to differ from one area to another due to different soil properties in various geographical regions in the country.

2.2.3 Pest and diseases

There are many pest and diseases that attack the potatoes and their number and type depend on the region. In sub- Sahara Africa, the common pests include nematodes, aphids, leaf miners and potato tuber moths. The diseases are such as bacterial wilt, late blight and viruses (Harahagazwe *et al.,* 2018). There are high incidences of late blight, bacterial wilt and viruses that affect potato yields in Eastern Africa. The high occurrence of the diseases in the region are attributed to the lack of good potato seeds, lack of knowledge to apply chemicals to control the diseases, lack of proper field sanitation, lack of crop rotation and low disease resistance in the grown varieties among other factors (Harahagazwe *et al.,* 2018). The presence of viruses and other diseases is highly attributed to the general lack of disease free potato seeds.

Potato late blight is the most important plant disease because it causes serious and high crop losses in most parts of the world (Lal *et al.,* 2018). Mostly, small-scale farmers use fungicides to manage late blight, but this practice has negative effects such as over reliance on pesticides,

poor human health and pollution of the environment. The management of late blight points at actions on different approaches, so any farmer that aims at the disease should consider using an integrated management approach to control this problem.

Bacterial wilt is the other major constraint in potato production. It is favoured by warm temperatures such like the ones found in the tropical and sub-tropical regions. The bacterium causes damage of the tubers which happen to be the part needed for food, selling and seed production (Charkowski *et al.,* 2020). The main effects witnessed are high yield losses, the tubers are not marketable and cannot be used as food. This eventually has economic implications on the farmer and the country. In a study carried out in seven counties in Kenya, a prevalence rate of 68.57% of the disease was reported (Kago *et al.,* 2016). Similar to the late blight, potato bacterial wilt does not have one general method that can effectively control it. An integrated approach is needed and dependent on some factors such as the distribution of the disease, the risk factors and the farmers understanding on the management of the disease (Uwamahoro *et al.,* 2018).

There are many viruses that infect potatoes in the world and their prevalence is dependent on the region. There are various viruses that affect the potato in Kenya such as Potato Leafroll virus, Potato, Potato virus A, Potato virus S, Potato virus M, Potato virus X and Potato virus Y (Onditi *et al.,* 2020). The use of clean potato seeds and resistant potato varieties are some of the approaches recommended to control the viruses. Some pests like aphids and whiteflies are known to spread the viruses and therefore they should be controlled in the potato fields (Kreuze *et al.,* 2016).

Nematodes have been known to cause considerable damage on the potatoes. Some of The common nematodes found in Kenya are *Meloidogyne incognita*, *M. javanica*, *Scutellonema unum*, *Rotylenchulus variabilis*, *Helicotylenchus dihystera* and *Paratrichodorus allius* (Njuguna & Bridge, 1998). Recently two potato cyst nematodes have been reported in the country, which are *Globodera pallida* (Mburu *et al.,* 2018) and *Globodera rostochiensis* (Mwangi *et al,* 2015). The nematodes damage the roots thus interfering with the uptake of water and nutrients.

2.3 *Globodera rostochiensis*

Globodera rostochiensis W. whose common name is Golden nematode, golden potato cyst nematode or yellow potato cyst nematode belongs to class *secernentea*, order *Tylenchida* and family *Heteroderidae*.

2.3.1 Description of *G. rostochiensis*

The eggs are always within the body of the female and not deposited in an egg sac. When the female dies, it is filled with eggs which contain second- stage juvenile (J2s) folded four times (CABI, 2021). Egg shell is hyaline with a smooth surface. The eggs measure 101-104 µm in length by 46-48 µm in width. The J2s are motile and vermiform in the soil but are swollen and sedentary inside the root. They measure 366-470 µm in length. The mouth has a strong stylet and the tail is pointed. On the body surface, cuticular annulations are prominent and have four incisures on the lateral field. The head bears 4-6 annules and is slightly offset and rounded. The stylet measures 21-33 µm long, knobs are rounded and slope backward slightly. Esophageal glands extend ventrally for about 35% of body length (USDA-ARS, 2014).

Third and fourth stage (J3s and J4s) Juveniles have no detailed description (Fatemy & Ghasemi 2018). They are swollen, sedentary and occur within the plant root. Adult females are swollen, sedentary and protruding from the root surface. The body measures 250-810 µm in diameter with a projecting neck. As the female develops into a cyst during its development, the colour changes from white to golden yellow as it matures. The head has one or two annules. The stylet knobs are rounded and stopped posteriorly. Median bulb is large and nearly spherical with well-developed valve. Esophageal glands are in broad lobe and often obscure. Excretory pore is near the base of the neck. The vulva which is a slight almost circular depression is found opposite the neck. The number of cuticular ridges on anal-vulval axis are 17-24 (Subbotin *et al.,* 2011).

The cysts (Figure 1) are similar to females in shape but their skins are tanned and organs are degenerated. They are actually a tanned sac which contains the eggs and is derived from some or all components of the dead mature female body wall (EPPO, 2017). Cysts are either found in the soil or attached on the roots. They are brown with a spherical or sub-spherical shape. Their length without the neck is 395- 495 µm and a width of 322- 442 µm. The neck measures 85-123 µm with a mean fenestral diameter of 17-21 µm. From anus to fenestra it measures 56.2- 76.8 µm with a Graneks ratio of 2.8- 4.4 (EPPO, 2017).

Figure 1: *Globodera rostochiensis* cysts

Males are present in the soil or coiled inside the cuticle of J4s. They are vermiform and motile, measuring 1100- 1300 µm long. Their copulatory structures are close to the end of posterior body. Their cuticular annulations are prominent and the lateral field has 4 incisures. The cephalic framework is heavily sclerotized. The head is slightly offset, hemispherical with 6-7 annules. The stylet is strong with prominent knobs that slope backwards slightly. Esophageal glands are narrow, ventral and terminate near the excretory pore. The testis is single and spicules have rounded tips that are unnotched. The tail is short, the length and shape is variable (Golden & Ellington, 1972).

2.3.2 Life cycle of *Globodera rostochiensis*

Eggs which are contained within the cysts are the persistent stage of the life cycle. Each cyst contains between 200 and 500 embryonated eggs. Eggs hatch to J2s and the hatching increases rapidly after plant emergence (Devine & Jones, 2003). The host produces secretions that stimulate J2s to hatch and also soil temperatures above 10ºC. The optimum soil temperature for hatching and development is 15 to 27ºC (Kaczmarek *et al*., 2014). For optimal root

penetration, the upper threshold temperature is 28ºC such that above this soil temperature, no nematode parasitization occurs.

The J2s penetrate the root behind the root tip after which they move through the root feeding on the cortex, endodermis and pericycle. The J2s then establish a permanent feeding site consisting of 'transfer cells' known as syncytia, which provides nutrients to the nematode (EPPO, 2004; Eves-van den Akker *et al.,* 2016). J2s undergo three molts before they can reach the adults stage. Sedentary female adults enlarge and burst through the root with the posterior body portion facilitating mating. Females exude sex pheromone to attract mates. The males which are vermiform and motile leave the roots to mate (Knoetze, 2014). Mating occurs within 50 days of J2 root invasion and the females continue feeding and retain their eggs within their bodies. When the female dies, she becomes a cyst changing the colour from white to yellow or gold and finally to brown. Cysts remain attached on roots or in the soil (Figure 2). The eggs can remain viable within the cyst for 30 years (Winslow & Willis, 1972). This is because the eggs are encased in the cysts and are protected from adverse weather in the soil for long even without the host (Coyne *et al*., 2018). This species normally produces one generation per year. This was confirmed by a study carried out in Quebec, Canada by Mimee *et al.* (2015). In the study the duration of the life cycle was affected by the prevailing temperature but the type of the soil did not have a significant effect. The development from hatching to adult, takes between 38 to 48 days. The ratio of sexes is possibly determined by food supply.

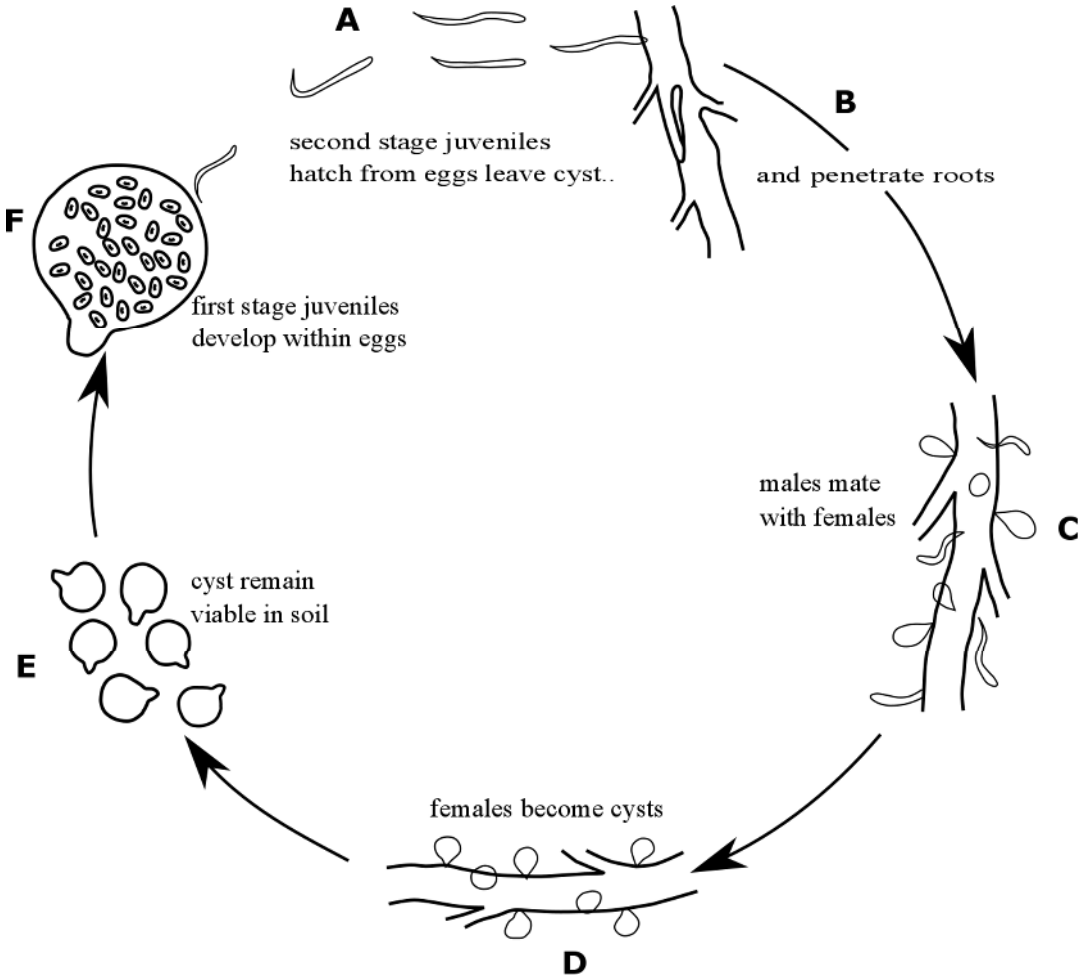

Figure 2: Life cycle of *Globodera rostochiensis*

Source: Scurrah (1981).

2.3.3 Host specification

The most important agricultural host of *G. rostochiensis* is *Solanum tuberosum* L. (potato). There are different pathotypes of the nematode that attack potato in different regions. Other than the potato, there are more than 150 known hosts of genus *Solanum* that allow the reproduction of the PCN. There are also other plants that belong to different genera that are host to the PCN (Sullivan *et al*., 2007). The nematode also occurs as a contaminant on non-host plants where the nematode is present. Among the known hosts there are those that are common in Kenya and include *S. nigrum* (black nightshade), *S. lycopersicum* (tomato), *S. melongena* (eggplant) and weeds such as *D. stramonium* (jimson weed) (Price *et al.,* 2021).

2.3.4 Transmission of *G. rostochiensis*

The J2s of *G. rostochiensis* can only move a maximum of one metre when locating a host (EPPO, 2004). The transmission of the cysts over long distances therefore depends on other

mechanisms such as wind, rain, flood water, contaminated propagation materials, agricultural tools, animals, machinery, non- host plants materials grown in infected fields (Blacket *et al.,* 2019). Since the nematode is soil borne, any item that comes in to contact with the infested soil can spread it to other areas. From this information, field hygiene is an important tool in combating the nematode transmission from farm to farm.

2.3.5 Symptoms *G. rostochiensis* on potato

The nematodes do not cause specific symptoms of infestation. What is noted first in the infested field is poor growth of plants (Figure 3) forming patches of chlorotic and wilting plants even during the rainy period (EPPO, 2004). The plants show small sized tubers that are fewer than in the healthy plants. This means there is yield loss during production. To confirm that the symptoms are caused by the nematodes, sampled soil contains cysts and the roots also have cysts or females attached on the surface especially on the tubers and the stolons. The plants also have reduced root systems leading to nutrient deficiencies and water stress. Plants dry up especially when affected by contaminant infections of other pathogens (EPPO, 2004).

Figure 3: *G. rostochiensis* hot spot in a potato field

2.3.6 Economic importance of *G. rostochiensis*

The actual damage of *G. rostochiensis* in potatoes vary from one region to another, but one thing that is in agreement is that it can be extensive. The damage is related to the virulence of the pathotype present, the number of eggs present per unit of soil and the soil temperatures in the area (Moreno *et al.,* 1984). For instance, Greco and Moreno (1992) carried out a study in Chile that showed that initial inocula levels of 12, 32 and 128 eggs/ g of soil led to reduction of yield by 20, 50 and 70% respectively during summer. An average loss of 2.75t/ ha per 20 eggs/g soil have been recorded in UK. The threshold for *G. rostochiensis* in the UK is 15 eggs /g soil, above which losses of as high as 80% can result (Brown, 1969). These infestation levels are much higher than the ones reported in Kenya of as low as 30 viable eggs per gram of soil by Mburu *et al.* (2020). However the presence of that nematode in about 80% of the potato growing areas is a threat to the potato production in Kenya.

Some important agricultural crops such as egg plants and tomatoes known to host the potato cyst nematodes. If the nematode spreads to the areas where the vegetables are produced, it would cause considerable damage. Tomatoes accounts for 20% of the total vegetables grown in the country (Horticultural Crops Directorate, 2016). They were valued at 13.6 billion shillings per year while the egg plants were valued at 356.3 million shillings in the year 2016. The contribution of these two vegetable crops to the country's cannot be underestimated, hence the need to protect them from the nematode. Since *G. rostochiensis* is a quarantine pest in most countries (EPPO, 2018), invasion of the nematode into an area or country, poses a regulatory restriction of marketing and export of products into other areas. Consequently, this would translate to a reduction in the per capita income of a country and also lower returns to the farmers.

2.4 Control measures

Various methods are used to manage the PCN (Bairwa *et al.,* 2017a). The methods are divided into three main categories namely; The biological control which entails use of predators of the nematodes, plant extracts, antagonistic fungi and bacteria and endophytic fungi and bacteria; Secondly, the cultural practices that include the use of crop rotation, soil amendments, green manures and physical soil treatments; and thirdly, the chemical control that involves use of nematicides (Bairwa *et al.,* 2017b). Other methods that are more modern include use of naturally resistant plant varieties and transgenic plants with resistant genes. When the methods are applied during the penetration by J2s, they control the damage of the plant to a great extent.

When the methods are applied during the other juvenile and adult developmental stages, they control the PCN population (Khalil, 2013). Some of the methods are discussed below.

2.4.1 Use of biocontrol agents

There are several fungi and bacteria that have shown antagonistic relationship with *G. rostochiensis*. Although most of them have been tested *in vitro*, there are a few that have been tested in the soil and have been effective (Mukhtar *et al.,* 2013). *Pseudomonas oryzihabitans,* a bacterium, was studied and tested as a biocontrol agent against *G. rostochiensis*. Its motility and efficacy was tested with respect to temperature. The *P. oryzihabitans* significally reduced invasion of J2s of *G. rostochiensis* in potato roots at 25^{o}C and 21^{o}C (Andreoglou *et al.*, 2003). Three biocontrol agents, *Pseudomonas flourescens, Purpureocillum lilacinum* and *Trichoderma viride* were tested against the cyst populations of *G. rostochiensis* and *G. pallida.* They all reduced the rate of cyst multiplication, ability of the J2s to penetrate the roos and the number of eggsin the cysts (Nagachandrabose, 2020). The effectiveness differed depending on three main factors, the rate of application, and the type of the agent and the method of application. The best perfomer when applied on the seed directly was *P. flourescens* while *P. lilacinum* was the best when applied directly on the soil.

Davide and Zorilla (1995) carried out experiments both *in vitro* and on farm which showed that a fungus *Paecilomyces litacinus,* significantly controlled the number of cysts in the soil of a potato farm infested with *G. rostochiensis*. In the same study, two other fungi, *Gliocladium roseum* and *Arthrobotrys cladodes* were shown to effectively control *G. rostochiensis* and also led to a significant increase in the potato yield. However, the effectiveness varied with method of application. Dipping tubers for 10 minutes in *P. lilanicus* suspension before planting had better nematode control than the soil- mix method. *P. lilacinus* was more effective (31.9- 50.8% cyst reduction) than *G. roseum* and *A. cladodes* (22.5-41.9% reduction).

The Kenya pest control product (PCPB) board has registered several bionematicides made from microorganisms such as BIONEMATON whose active ingredient is *Paecilomyces lilacinus* (PCPB, 2018). However, there have been concerns over their use such as their adaptability to other areas away from their original habitat, their influence on other microbes in the soil due to their interactions, ability to be reproduce them commercially and also compatibility with other production methods (Montesinos, 2003).

2.4.2 Quarantine

Quarantine is the restricted movement of infected materials from infected areas to uninfected areas. Different regions and countries apply different regulatory methods against the introduction of nematodes in their regions. The overseeing body for plant protection in the regions of the world is the Region Plant Protection Organisation (RPPO). In African region, the organization is called the Inter- African Phytosanitary Council (IAPSC) (FAO, 2021). In Kenya, KEPHIS is mandated to carry out quarantine activities for materials getting into the country and within the country (Ochilo *et al.,* 2018).

In regions under EPPO, fields in which seed potatoes or rooted plants for export are grown, the planting materials and soil samples are inspected before and after harvesting potato crop to ensure that incidences of *Globodera* species are closely monitored (EPPO, 2009). Quarantine is necessary but expensive especially where there are no accurate ways of identifying the nematode (MAFF, 2000). However, it is widely used to avoid the introduction and spread of the *Globodera* species. In some countries, computerized methods are used to screen against the nematode during importation, since trade is one of the major ways through which the nematode is spread (Been & Schomaker, 2000). According to EPPO 2014, many countries in the EU have implemented data systems to keep records on the various soil sampling results on PNC and also use automated system (carousel) to extract the cysts.

When planting, materials and soils are screened against the nematode; they ensure that the fumigants are not used unnecessarily in the field. The fumigants are highly toxic and expensive and therefore only used when extremely necessary (Been & Schomaker, 2000). If quarantine measures are applied strictly in all countries during importation of potato seeds and also within the country, the spread of *Globodera* can be arrested to some extent.

2.4.3 Chemical control

Chemicals used to control nematodes are collectively referred to as nematicides. This method is expensive to the potato producers and also the effects on the environment and users are adverse (Sorribas *et al.*, 2005). Nematicides are often not acceptable as compared to other pesticides due to their high cost, phytotoxicity and negative effects on the environment. Nematicides are highly toxic with very low LD_{50} values. These put the people who are exposed to the chemicals during application at risk from exposure. Skilled operators are required so that they can take adequate safety precautions (Soltani *et al.,* 2013). Application of nematicides just

before harvesting can also pose a risk to the consumers of the products. Nematicides also affect non- target organisms in the soil which are nematode competitors, predators and parasites (Chitwood, 2003). For instance, methyl bromide can eliminate mycorrhizae, while aldicarb treatment can decrease the bacteria in the soil thus interfering with general plant growth. It also affects the distribution and structure of the soil communities. Most of the nematicides used in Kenya are restricted to large farms especially the flower farms and Delmonte Kenya. Some of the restricted nematicides in the farms include Oxamyl 250 g/ l which is should be used on pineapples and ornamentals, Fosthiazate 100 g/. l on roses and Carbofuran m/ m. Some nematicides were banned in 2009, such as Benomyl above 7%, Carbofuran above 10%, Thiram above 15%, Methyl bromide and all tributylin compounds (PCPB, 2018).

2.4.4 Trap crops

These are plants that have natural ability to attract pests and pathogen for feeding and egg laying and also have other attributes that enable them to serve as a sink for the pest or pathogens (Shelton & Badenes – Perez, 2006). They mostly prevent the survival of the target pest by interfering with its development. The trap crop may be planted around field perimeter or interplanted with the main crop. In the UK, potatoes are grown as trap crops during the off season (Halford *et al.*, 1999). Close monitoring is required for potatoes from planting date to ensure no new eggs are formed by destroying the crop 6 to7 weeks after planting. If well timed, *G. rostochiensis* can be reduced by more than 80%. Other crops that have shown effective reduction in PCN include *Solanum sisymbriifolium* (can reduce PCN by upto 75%) (Scholte, 2000).

However, Clayton *et al*. (2008) observed that the cost of using *S. sisymbriifolium* as a trap crop was much higher than the use of conventional nematicide. This calls for researchers to consider cost before recommending a trap crop. *Solanum nigrum* (Black nightshade) is a common annual plant and is considered as a weed in most countries (but traditional vegetable in Kenya). The tests done in UK in 2011using *S. nigrum* resulted to great reduction in PCN (80.1-90.6%) (Sparkes, 2013). Other plants of genus *Solanum* such as *S. scabrum* and *S. villosum* have shown that they can be tried as trap crops (Scholte, 2000). Unlike crop rotation, trap crops ensure that the cyst hatch just as in the case of potatoes but doesn't support its development. Most of them secrete chemical substances that stimulate hatching but the J2s are either unable to penetrate or they fail to form the feeding sites on the trap crop's roots (Fatemy & ahmarimoghadam, 2019).

2.4.5 Crop rotation

Crop rotation is a systematic approach where different crops are grown in succession on the same land. Crop rotation breaks the pest and disease life cycle bringing its density under check (Lima *et al.,* 2018). When designing a crop rotation programme, it's a general rule of thumb to rotate crops that are not related to each other (Katsvairo *et al.,* 2006). There is need to identify alternative hosts of the nematode and non- hosts to make the programme more effective. Rotation intervals range between 4 and10 years in different countries (Lima *et al.,* 2018). Plants used can either be enemies that release substances in the soil that negatively affect the nematodes or they can trigger them to hatch but fail to provide suitable nutrients or habitat for nematode development, that is trap crops. Rotational plants can also be either weeds that are used as green manure or non- host crops. In absence of host crop, there is some degree of spontaneous hatching and in-egg mortality that leads to a reduction in the number of viable eggs in a field. A field study carried out in Ireland showed a 57% and 40.3% decline of *G. rostochiensis* populations during the first and second year of crop rotation respectively (Devine *et al*., 1999).

Crop rotation alone cannot be very effective more so due to limited arable land available for plant production (Lopez- Lima *et al.,* 2013). It is therefore recommended that integrated methods are used rather than crop rotation alone. Crop rotation is integrated with use of manures, resistant varieties and cover crops (Neher *et al.,* 2019). The basis of rotation is that, without the host plants, there would be a significant reduction in viable eggs in a PCN cyst. The longer the interval, the more effective the method is, although this is influenced by environmental factors, such as soil type (FERA, 2010).

2.4.6 Use of transgenic plants

Transgenic plants are the ones whose DNA is modified mostly by introducing a new trait to the plant that does not naturally occur in the species. One or more genes are artificially inserted in to the plant (Rani & Usha, 2013). In the view that the global population is increasing by day, there has arisen a need to increase the yield and other better traits in plants so as to feed the growing population. Use of transgenic plants has several advantages and disadvantages (Davies, 2007). Some of the benefits of genetically modified plants are development of plants that are resistant to herbicides, insects, viruses and pests. The plants also have high nutritional values, therapeutic proteins, have reduced negative environmental impact and withstand drought conditions. However the plants are thought to have negative effects such as damage of

natural environment, ill human health such as allergic reactions, emergence of super- pests, loss of diversity and uncertainties in their regulations (Fontes *et al.,* 2002).

Studies have been done on effects of transgenic potatoes on the potato cyst nematodes. In a study done by Green *et al.* (2012), a transgenic potato effectively suppressed the invasion of J2s in to the plant. It had a peptide and a cysteine proteinase inhibitor that gives the plant resistance to the pest nematode. It was advantageous since it had no effect on the other nematode communities found in the soil. In another study, two transgenic potato varieties, Desiree and Asante were tested under field conditions against *G. pallida.* They both showed a high resistance to the nematode of 70 ± 9% and 85.± 3% respectively (Urwin *et al.,* 2001). In some countries transgenic plants have been used for over 20 years (Dandurand *et al.,* 2019). They are viewed as good way of improving food security and having plants with better survival characteristic.

2.4.7 Uses of resistant varieties

Development of resistant varieties has been explored as a management practice against *G. rostochiensis*. The method is environmental friendly with no adverse effects on soil microbes, human, animals and plants. Use of resistant varieties reduces the reproduction of the nematode in the soil due to lack of appropriate host leading to its elimination with time (Green *et al.,* 2012). The use of resistant cultivars is opted for in the control of PCN as compared to the use of the nematicides. Other than reduced environmental risks, the cultivars resist the multiplication of the nematodes and minimize crop damage. This results to high potato yields (Blok *et al.,* 2018). A study carried out in Quebec, Canada, showed that crop rotation with resistant potato varieties was very effective in decreasing the population density of *G. rostochiensis* in soil (Belair *et al.*, 2016). In a single year with resistant variety, the population of the nematode reduced by 62- 92% and in three consecutive years, the overall population reduced to around 95.5%. This was far much high as compared to a 30% reduction recorded when non- host crop (corn) was used during a crop rotation programme

The resistant varieties are mostly used together with crop rotation. In Germany, resistant varieties are planned in a 6- year rotation while in Denmark resistant varieties are grown in two consecutive years (different varieties each year) to reduce their re-occurrence (EPPO, 2014). However, the use of resistant varieties in an infested field over time poses a risk of selecting a virulent nematode population especially where unknown mixtures of species and pathotypes

are present together (Atkinson *et al.,* 2001). To avoid the problem, different resistant and tolerant varieties are alternated. Most varieties show moderate resistance but in New Zealand there are varieties with high resistance to PCN like 'Karoka' (Anderson *et al*., 1993). From the AHDB (2021) potato variety database in UK, some of the varieties that show resistance to *G. rostochiensis* are Paramount, Premiere, Pixie, Banba, Dairy, Fambo and Fontane. In Czech Republic, 132 varieties of potatoes have been recorded being resistant to *G. rostochiensis* (SRS- Czech Republic, 2011). Also in Netherlands, there are many varieties that are resistant to *G. rostochiensis* and are used to keep the nematode under check (NIVAP, 2011).

In Russia, an experiment carried out using resistant varieties in the field, showed that resistant varieties stimulated the release of larvae from the eggs into the soil. Over 4 years they helped to achieve full control of soil infestation with *G. rostochiensis* pathotype Rol (Anosova and Safronova, 2001). In Serbia, known resistant and susceptible potato varieties were tested for their effect on population of golden cyst nematode in infested fields by Basic (2010). The population of *G. rostochiensis* was reduced and higher yields obtained by growing resistant varieties. In the second year of investigation, the final population density was 3.5 times lower than the first year and the yield at 25:7 - 29.1 ton/ha. These results from the tested fields are useful to producers of potatoes in infested areas when choosing potato varieties, in order to eradicate the nematode. Further research has shown that the use of resistant varieties such as transgenic potatoes, disrupts the chemoreception of potato cyst nematodes without affecting the non- target nematodes and other soil organisms (Green *et al.,* 2012). The resistant varieties therefore have been shown to manage the nematodes without interfering with the soil quality which is a great concern when other methods are used (Neher *et al.,* 2019). Since potato production is intensive in Kenya, the use of resistant varieties should be integrated with other pest management methods so as to manage the spread of the potato cyst nematode (Muthoni *et al.,* 2017).

2.4.8 Use of plant extracts

Use of natural compounds to manage pests and diseases is an alternative method to use of chemicals. Natural compounds are environmental friendly and most of them are less toxic as compared to synthetic chemicals while they can be effective in control of the target organism. (Wiratno *et al.*, 2009). The neem leaf and garlic bulb extracts were evaluated by Agbenin *et al.* (2005) against *Meloidogyne incognita* in tomatoes. The two extracts were reported to reduce the nematode infection index significantly. Plant extracts of *Nicotiana tabacum* and *Veratrum*

album mixed with commercial neem oil at 0.5% and 0.1% respectively were investigated against *G. rostochiensis* by Trifonova and Atanasov (2011). The treatment with the above extracts resulted to improved plant growth and increased yield of potatoes from 0.6- 13.2%. Treatment with neem oil alone resulted to a 0.9% yield increase. The *G. rostochiensis* population density also decreased significantly with the best reduction being obtained in the application of *Nicotiana tabacum* and neem oil 0.5% (77.7% reduction) and *V. album* and neem oil treatments (77.8% reduction) (Trifonova & Atanasov, 2011).

Another plant that has been used to manage *G. rostochiensis* is *Medicago sativa* L (alfalfa). In a study carried out in Italy, the roots and dry top materials from *M. sativa* were used in potting mixes infested by *M. incognita* and *G. rostochiensis*. All amendments reduced root and soil population densities of the two nematode species as compared to the controls (D' Addabbo *et al.,* 2010). From the experiments, J2s of *G. rostochiensis* were the most susceptible to *M. sativa* saponins as 40-54% mortality rates occured between 4 and 8h at 125µg ml^{-1} and 68-91% after 24h exposure at 250-1000 µgml^{-1}. Saponins of *M. sativa* were particularly toxic to *G. rostochiensis* where the saponins interact with cell membrane causing changes in cell permeability. Plant extracts of *D. stramonium* were tested against *M. javanica* in melons at different concentrations. In the laboratory, the mortality rate of juveniles was as high as 97%. In the field the population density of the nematodes was far much lower in the vines treated with *D. stramonium* powder as compared to the control (Umar & Ngwamdai, 2015). In Kenya there are bionematicides used against root knot nematodes. Bionematicides allowed by the Pest Control Board include Bionematon 1.15 % WP and Azidirachtin 0.3 % made from neem (PCPB, 2018).

2.4.9 Soil amendments

Soil amendments through application of organic matter and green manure have been shown to decrease the nematode pest populations. This is due to improved soil structure and fertility, alteration of plant resistance, release of nemato- toxins and increased population of antagonistic fungi and bacteria (Walker, 2004). As a result of this findings, many studies have been carried out to investigate the effect of the soil amendments on various nematodes. Selected brassicas were used as green manure in soil containing encysted eggs of *G. pallida*. The three effective brassica lines were found to have isothiocyanate- producing glucosinolate that caused over 95% mortality of encysted eggs (Lord *et al*., 2011). This shows that once identified, the brassicas can be incorporated into the soil during potato planting to reduce the incidences of

PCN in the soil. Other than brassicas, other plants such as neem, castor oil plant and marigold with nematicidal effects have been used for green manuring and have proved effective.

The population of eggs of *G. rostochiensis* reduces significantly when organic matter is applied. High nitrogen contents in the manures have nematicidal effects. In a survey carried out in East Slovakia by Renco *et al.* (2011) under field conditions, compost manure of different origins had suppressive effect on the reproduction of *G. rostochiensis.* The number of eggs per cyst also reduced depending on the rate of manure application. Poultry manure contains significant quantities of nitrogen, phosphorous, potassium, calcium, magnesium and micronutrients (Riegel & Noc, 2000). Application of chicken litter as soil amendment has shown that it reduces the population densities of plant- parasitic nematodes. This is because nitrogen contained in chicken litter is in form of uric acid that can quickly be converted to ammonium, which has nematicidal effects.

In conclusion, there are many methods that are used to manage *G. rostochiensis* where it occurs. However, there are no documented methods that are used to manage the nematode in Kenya currently. This study therefore aimed at using environmentally friendly methods to manage the nematode that can easily be incorporated with other farming practices. This will contribute towards improved potato production, living standards and national food security.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

This study was done in Njoro and Molo Sub Counties, Nakuru County, Kenya (Figure 4). The areas are known for high potato production in the county (MoALF, 2016). Potatoes are mainly grown on the cooler areas of the Sub Counties where other crops such as carrots, peas, beans maize, vegetables, wheat, barley and pyrethrum are grown. Among the crops, potato is considered as the highest earning crop with households allocating 25- 100% of arable land to potato production (Mwaniki *et al.,* 2016). Potato production range between 5.9- 26.8 ton/ ha, in low and high altitude areas respectively, giving an average of 14.5 ton/ ha (Mwaniki *et al.,* 2016). The popular potato varieties are Shangi, Tigoni, Kenya Mpya, Kenya Karibu, Nyayo, 'Dera Mwana', Mwezi Moja, 'Thima-thuti' and Sherekea. Other than crops, livestock like cattle, sheep and poultry are reared.

3.1.1 Njoro Sub- County

The area lies between the eastern edge of Mau forest complex and Lake Nakuru National Park, giving it diversified climatic conditions ranging from semi- arid (Lare) to upper highlands in Mau Narok and Mauche. It stands at an altitude of between 1600 m- 2894 m above sea level. The temperatures range between 6-27^{o} C while annual average rainfall is between 600- 1800 mm received two seasons per year (MoALF, 2017). The area has about 46,004 households and 33,466 farm families. In high potential areas, land holdings are mainly on small scale with average land holding of 0.77ha. (NCIDP, 2013). The potatoes are mostly grown on plots separate from other crops such as peas, maize, beans and wheat. Soils are volcanic, well drained, moderately deep and brown to dark brown, very friable loam to sandy clay (MoALF, 2017).

3.1.2 Molo Sub- County

The sub- County covers an area of 478.7 sq. km out of which 69.6% is arable land and the rest is under forest. It has four wards namely Molo, Turi, Elburgon and Mariashoni. It receives an average rainfall of between 800- 1681 mm per year with temperatures between 9- 28^{o} C. The soils are diverse and include sandy loams, sandy clays, clay loams and mollic andosols. There are 30,783 households with 27,375 farm families with an average landholding of 1.3 ha. Other than potatoes, other important crops grown in the area include wheat, maize and beans (MoALF, Molo, 2017).

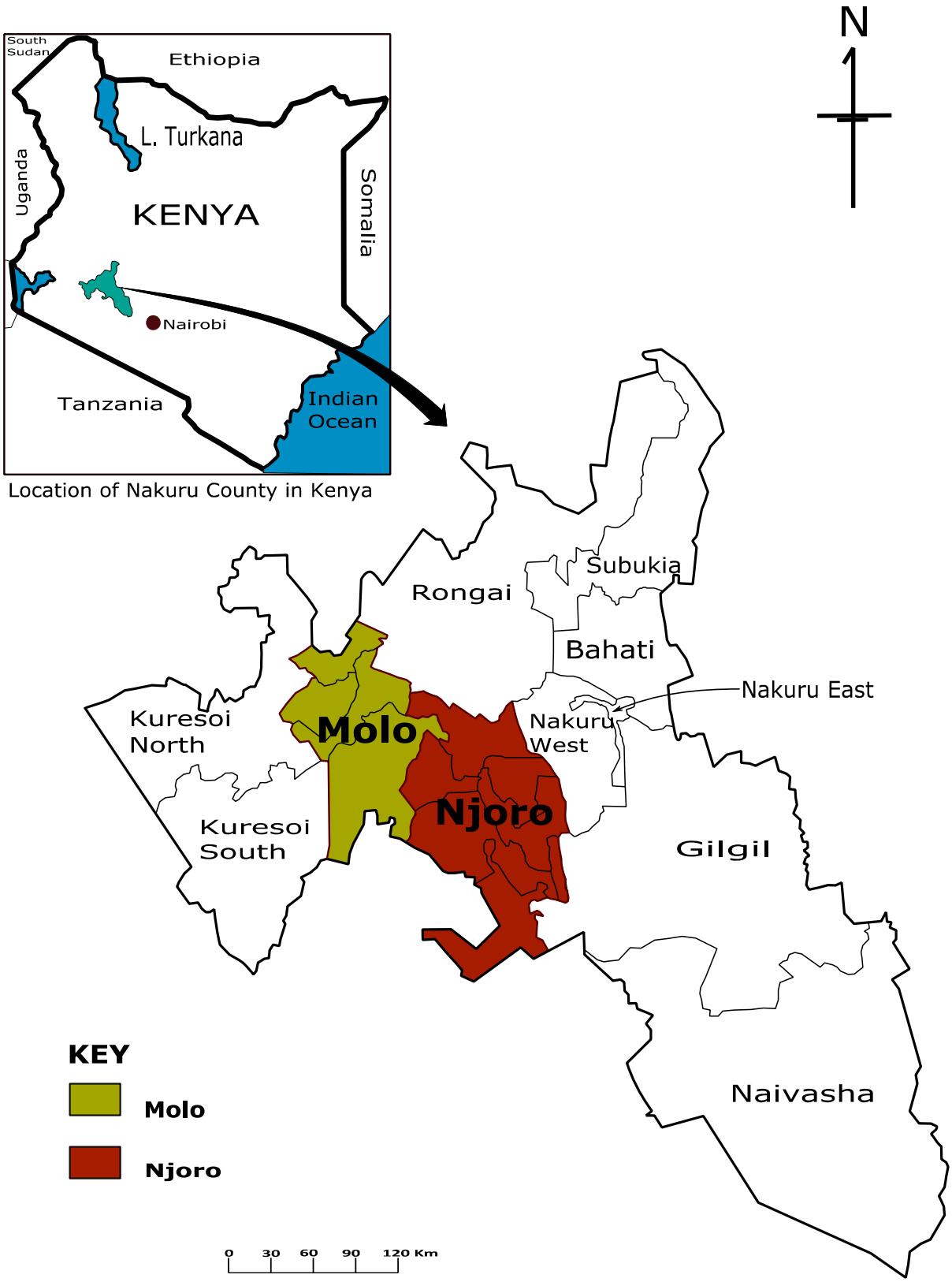

Figure 4: Map of study area

Source: NCIDP, (2013)

3.2 Cyst sampling

Cysts were obtained from naturally infested fields in Njoro and Molo sub-Counties. The sampling was purposive, where 'hot spots' were identified in the infested potato fields with assistance from the agricultural extension officers. Hot spots are characterized by patches in the potato field with plants that show stunted growth, chrolotic leaves and small in size (Turner, 2008). A shovel was used to collect soil to a depth of approximately 30 cm in the 'hot spots.' The soil was placed in plastic bags and taken to the laboratory.

3.3 Cyst extraction and inoculum preparation

The soil was air dried for 2 weeks by spreading it on trays at room temperature to maintain cyst viability (EPPO, 2013). Drying of the soil ensures that the dry cysts float on water. Extraction of the cysts from the soil was done using bucket method and Fenwick can (Fenwick, 1940) at ICIPE, Nairobi. The water collected contained few floating organic debris and the cysts. The water was filtered using milk filters before leaving the cysts and debris to dry at room temperature. The cysts were later hand-picked using forceps under a stereomicroscope in Egerton University laboratory and put in dry vials. They were preserved at 4^{o}C and used for hatching and as inoculum for the pot experiments.

3.4 Soil sterilization

The farm soil used for pot experiments was obtained from Field 3 of Egerton University, Njoro, Kenya. The soil was heat sterilized using a modified procedure as described by Abdullah (2010). It was autoclaved for 30 min at 80^{o} C under a pressure of 105 Pa. in the Biological Sciences laboratories. The soil was allowed to cool and stored in sacks for six weeks before planting to regain stability.

3.5 Screening test varieties for resistance against *G. rostochiensis*

In this study the EPPO standard method for testing potato resistance was used, where a standard susceptible control was included in the experiment (EPPO, 2006). The population of the *G. rostochiensis* in the standard was compared with the population in the test varieties. This was used to calculate the Relative Susceptibility (RS) and the Reproductive Factor (RF) of the test varieties.

Tubers of six commonly grown varieties were sourced from ADC Molo and used for screening for resistance. These test varieties were Shangi, Tigoni, Kenya Karibu, Sherekea, Dutch Robjin

and Kenya Mpya. Desiree variety was used as the standard susceptible control variety (EPPO, 2006). The tubers were planted in plastic pots measuring 13 cm diameter (Figure 5). For each pot 1000 g of sterilized soil and 10 g DAP mixture weighed separately with a weighing balance was used. The soil was put in the pots half-way and then 25 cysts (PI) was placed on the soil to achieve a population of about 4,000 eggs per pot. Single tubers sprouted before planting were placed per pot and then covered with rest of the soil. Each pot was labelled according to variety and date of planting. Four replicates per variety were placed on raised benches in a complete randomized design (CRD). The experiments were carried out in a greenhouse at Egerton University. The pots were watered regularly and the necessary management practices carried out. Harvesting was done when plants started flowering (10 weeks old).

Figure 5: Potted potato in the greenhouse

3.5.1 Evaluation

Cysts per pot were extracted, counted and recorded as explained in section 3.3. The mean per variety from all the four replicates were calculated to give the final population (PF). The PF and PI were used to calculate the reproductive factor (RF) and the relative susceptibility (RS) of the test varieties (EPPO, 2006).

3.5.2 Reproductive factor (RF)

$$RF = \frac{Final\ nematode\ population\ (PF)}{Intial\ nematode\ population\ (PI)} \qquad 1$$

If RF is >1, the variety is susceptible.

<1, means the variety is resistant.

3.5.3 Relative susceptibility (RS)

$$RS = \frac{PF\ Test\ variety}{PF\ Standard\ variety} \times 100 \quad 2$$

The standard score for RS was as below.

Relative Susceptibility (%)	Score
< 1	9
1.1 – 3	8
3.1 – 5	7
5.1 - 10	6
10.1 - 15	5
15.1 - 25	4
25.1 - 50	3
50.1- 100	2
> 100	1

9 – Represent the highest level of resistance.

3.6 Effect of chicken manure on cyst population

Chicken manure was sourced from a local farmer. The manure was mixed with the sterilized soil at a rate of 1:2 w/w manure to soil. The standard susceptible variety Desiree, was planted in plastic pots measuring 13cm in diameter. For each pot 750 g of the mixture was used. The pots were first filled half-way with the soil- manure mixture after which 25 cysts were added. One spouted potato tuber was placed per pot, covered with the rest of the soil and then labeled. Four replicates were set and a control with unamended soil in four replicates was included. The pots were arranged in CRD in the green house. Management practices were carried out until the 10^{th} week when harvesting was done.

3.6.1 Evaluation

Cysts were extracted from each pot using Fenwick method (Fenwick, 1940). They were counted and recorded. The results of treated pots were compared to those of the control.

3.7 Effects of aqueous extracts of *D. stramonium* on J2 mortality

D. stramonium L. is a plant that belongs to Solanaceae family and is widely distributed in the world. The stem is herbaceous, branched and slightly hairy. The leaves are big with the colour

ranging between deep to pale green. It seeds are black, kidney shaped and found within a hard pericarp when dry. It has a tap root system that supports the growing plant (Tapfuma *et al.,* 2019). The plant extracts and paste have been used over a long time to treat various human diseases including inflammation, sciatica, rheumatism, swellings, wounds, fever, ulcers and toothache (Gaire & Subedi, 2013). Various studies show that the external application of the plant products does not necessarily have negative effects, but oral and systemic intake may lead to severe nervous breakdown (Soni *et al.,* 2012). The plant has many metabolites that make it diverse in application. These metabolites are mainly alkaloids that can be grouped in to two, major and minor. The major alkaloids are hyoscyamine and scopolamine. The minor ones include tigloidin, aposcopolamine, apoatropin, hyoscyamine N- oxide and scopolamine N- oxide 17-20 (Shagal *et al.,* 2012). These compounds and many others are tested for their pharmacological activities so as to be used in different fields.

Plant extracts from leaves, roots and seeds of *D. stramonium* (Figure 6) were used in this study. The plant was positively identified using the plant anatomy and confirmed by a botanist in department of biological sciences of Egerton University. The plant parts were first prepared for use.

Figure 6: *D. stramonium* plant

3.7.1 Collection and preparation of plant material

Fresh whole Jimson weed (*D. stramonium*) plants (including root, leaves and stems) were collected from Egerton University. The plants were thoroughly washed in running tap water and then finally washed with distilled water (Nandakumar *et al*., 2017). The plant materials were then divided into leaves and roots. The seeds were collected from dry fruits of the plant in the same area. Each part was treated as described below;

Leaves extract

The leaf extract was prepared using a modified method described by Umar and Ngwamdai (2015). A 100 g of fresh *D. stramonium* leaves was cut and blended using a normal blender in 300 ml of distilled water and then allowed to settle in the refrigerator for 24h. The extract was centrifuged at 5000 rpm for 5 minutes after which the supernatant was passed through a filter paper (Whatman No 1) to obtain a clean extract. The filtrate was designated as crude extract (100%) and stored at .4 ^{o}C for not more than one week before use. During juvenile mortality test the crude extract was diluted into 50, 25 and 12.5% using distilled water.

Roots extract

The root extract was obtained using a modified method as described by Doughari and Manzara (2007). Medium sized roots of *D. stramonium* weighing 300 g were cut into small pieces and blended in 500 ml of water. The extract was centrifuged at 3500 rpm for 5 minutes and then filtered using Whatman No. 1 filter paper. The filtrate was designated as the crude extract (100%) and stored in refrigerator at 4 ^{o}C for not more than a week awaiting use. During the juvenile mortality test the crude extract was diluted into 50. 25 and 12.5% using distilled water.

Seeds extract

Tis extract was prepared according to a modified method as described by Aho and Agunwamba (2014). The dry *D. stramonium* seeds (10 g) were blended in 100 ml of water. The extract was filtered using whatman No 1 filter paper, after which it was centrifuged at 3500 rpm for 5 minutes and the supernatant collected. It was stored at 4 ^{o}C as crude extract and used for evaluation within one week.

3.7.2 Hatching of *G. rostochiensis* eggs

Cysts were extracted from soil collected from PCN infested farms in Njoro and Molo sub-counties, Nakuru County, Kenya, using Fenwick floatation method at ICIPE, Nairobi

(Fenwick, 1940). The root exudate were obtained from intact roots since they more closely reflect what nematodes may be exposed to in the rhizosphere (Wang *et al.,* 2018). Root exudate of *Solanum scabrum* (African nightshade) was used to stimulate hatching of the eggs. The exudate was obtained by first saturating pots containing one month old *S. scabrum* with distilled water. Then about 200 ml of distilled water was passed through the pots and the leachate collected in a container (Fenwick, 1949; Scholte, 2000). The leachate was centrifuged at 3500 rpm for 5 minutes and then used to stimulate hatching. Fifty cysts were pre-soaked for one week in distilled water after which they were exposed to the exudates.

3.7.3 Juvenile mortality test

The seed, leaf and root extracts at 12.5%, 25%, 50%, and 100% concentrations were dispensed in volumes of 5 ml in separate Petri dishes, using a 5 ml syringe. A control containing distilled water was included (Umar & Ngwamdai, 2015). Approximately twenty (20) freshly hatched *G. rostochiensis* J2s suspension were added to each of the Petri dishes containing the extracts using a micro pipette. Each dilution level had four replicates arranged in CRD on the laboratory benches. The number of dead J2s were counted at 24, 48 and 72 hour intervals (Tables 1). The dead J2s were identified by assumption of straight (I) shape and lack of motility after probing with a needle.

3.8 Data analysis

The significance of the varietal cyst population differences were analyzed using analysis of variance (ANOVA) and their means compared using LSD. The means of the cyst population in the soil treated with chicken manure were compared with those of the control using t- test. The mortality data was corrected for natural mortality using Schneider- Orelli's formula (Püntener, 1981). Comparison of mortality rates of the juveniles in different extracts was analyzed using two- way ANOVA and the means compared using LSD. The mean mortality percentages for each extract per day were represented graphically. All the hypotheses were tested at 95 % level of significance.

Other analysis that were done include the calculation of the Reproductive Factor (RF) and the Relative Susceptibility (RS) of the test varieties using equations1 and 2 in section 3.5.2 and 3.5.3 respectively.

CHAPTER FOUR
RESULTS

4.1 Screening common potato varieties for resistance against *Globodera rostochiensis*

The study revealed that all the six tested potato varieties were susceptible to the PCN (RS scores of 2 and 3). However the level of susceptibility differed among the varieties (Table 1). There was significant difference in the mean of *G. rostochiensis* population among the test varieties ($F(5, 18) = 6.138$; $P = 0.002$). The least susceptible varieties were Tigoni and Shangi with a mean population of 101.25 ±31.19 and 96.25 ±37.05 cysts respectively. Sherekea and Kenya Karibu had the highest cyst population (186.25 ± 50.23 and 220.00 ±44.91 cysts respectively). Kenya Mpya and Dutch Robjin had moderate susceptibility. The reproductive factors (RF) of all the test varieties were above 3.85.

Table 1: Reproductive Factor and Relative Susceptibility of the test varieties

Variety	Cyst Population	Reproductive Factor	Relative Susceptibility (%)	Susceptibility Score
Shangi	96.25 ± 37.053	3.85	34.2	3
Dutch Robjin	161.25 ± 32.755	6.45	57.3	2
Tigoni	101.25 ± 31.192	4.05	36.0	3
Kenya Mpya	162.50 ± 33.789	6.50	57.8	2
Kenya Karibu	220.00 ± 44.907	8.80	78.2	2
Sherekea	186.25 ± 50.229	7.45	66.2	2
Desiree	281.25 ± 34.970	11.25		

European Plant Protection Organization, % susceptibility compared to susceptible control. 1-9 scale; 9=<1%, 8=1.1-3%, 7=3.1-5%, 6=5.1-10%, 5=1-1-15%, 4=15.1-25%, 3=25.1-50%, 2=50.1-100, 1=>100%.

4.2 Effect of chicken manure on the population of *G. rostochiensis*

The application of chicken manure in Desiree potatoes inoculated with *G. rostochiensis* cysts, resulted to a significantly lower nematode population ($t(6) = 4.138$, $p < 0.006$), as compared to the susceptible standard control, the Desiree variety (Table 2). The populations in the amended and unamended soils had a mean difference of 145 cysts translating to 51.60% reduction. The reproductive factor (RF) of the treated variety was 5.44 compared to the untreated variety at 11.25.

Table 2: Effects of chicken manure on *G. rostochiensis* cyst population

Variety	Treatment	N	Mean
Desiree	None	4	281.25± 34.97
Desiree	Chicken manure	4	136.25± 60.74

4.3 Effect of aqueous extracts of *D. stramonium* on J2s

This study evaluated the effect of different levels of dilutions of *D. stramonium* root, leaf and seed extracts on the mortality of *Globodera rostochiensis* J2s. The cysts started hatching. 5-6 days after incubation. The mortality of J2s was significantly influenced by the type of extract, levels of dilution of extract and period of exposure (Table 3- 5). The control mortality was recorded as 2.5%, 12.5% and 30% after 24, 48 and 72h of exposure respectively, these results were used to correct the mortality data.

4.3.1 Nematicidal effects of *D. stramonium* extracts at 12.5 % dilution

A comparison of the leaf, root and seed extracts on the mortality rate of J2s at 12.5% dilution, showed that root extract had the highest effect (M=64.10 ± 13.24) while seed and leaf had mean mortality of M=29.49 and 16.67% respectively (Table 3). Similar pattern was observed after 48 hours of exposure with root having the highest effect on mortality (M=72.60 ± 7.75) followed by seed extract at M=53.43 ± 13.04) while leaf extract had the least effect on mortality on J2s (M=27.40 ± 9.36%). No significant differences were found among the three extracts at 12.5% after 72 hours exposure (F (2, 9) = 1.909, p=0.204.

Table 3: % Mortality rate of J2s on application of *D. stramonium* extracts at 12.5 % dilution at 24 hours interval

Extract	24h	48h	72h
Seed	29.49 ± 8.76 [a]	53.43 ± 13.04 [a]	85.72 ± 7.38
Root	64.10 ± 13.24 [b]	72.60 ± 7.75[b]	77.14 ± 4.67
Leaf	16.67 ± 2.56 [a]	27.40 ± 9.36[c]	82.86 ± 6.60
	F(2,9)=27.940, p=0.000	F(2,9)=19.441, p=0.001	F(2,9) =1.909, p=0.204

Values are expressed as mean ± SD of four replicates. Mean values within a column with common letter are not significantly different (P = .05) according to LSD test.

4.3.2 Nematicidal effects of *D. stramonium* extracts at 25 % dilution

At a dilution of 25%, LSD procedure indicated that root and seed extract had same effect on mortality of J2s (p=0.390) while leaf extract had significantly lower effect on mortality of J2s after 24 hours of exposure (Table 4). Similarly, the difference in the effects of root and seed extracts on mortality were not significant at 95% after 48 hours of exposure (p=0.418) and the leaf extract had significantly lower effect (M=31.51 ± 9.49). The effects of the three extracts after 72 hours of exposure were not significantly different. It thus shows that period of exposure is a critical variable influencing the nematicidal effect of plant extracts on J2s.

Table 4: % Mortality rate of J2s on application of *D. stramonium* extracts at 25 % dilution at 24 hours interval

Extract	24h	48h	72h
Seed	82.05 ± 8.88[a]	89.04 ± 7.75[a]	94.29 ± 8.08
Root	75.64 ± 7.70[a]	83.56 ±10.01[a]	95.72 ± 8.57
Leaf	29.49 ± 12.82[b]	31.51 ±9.49[b]	88.57 ± 4.67
	F(2,9)=32.631	F(2,9)=48.341	F(2,9)=1.068
	P=0.000	P=0.000	P=0.384

Values are expressed as mean ± SD of four replicates Mean values within a column with common letter are not significantly different (P = .05) according to LSD test.

4.3.3 Nematicidal effects of *D. stramonium* extracts at 50 % dilution

At a higher dilution of 50%, root and seed extracts resulted in higher mortalities than leaf extract. Root and seed resulted in 100% mortality after 24 hours exposure. Leaf extract had mean mortality of 42.31, 78.08 and 97.05% after 24, 48 and 72 hours exposure respectively (Table 5). A small proportion of J2s (2.85%) survived even after being exposure to 50% leaf extract for 72 hours as opposed to root and seed extracts which eliminated all the J2s after 24 hours exposure at 50% dilution.

Table 5: % Mortality rate of J2s on application of *D. stramonium* extracts at 50 % dilution at 24hours interval

Extract	24h	48h	72h
Seed	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00
Root	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00
Leaf	42.31 ± 4.91^{b}	78.08 ± 7.75^{b}	97.05 ± 3.30
	F(2,9)= 551.890	F(2,9)=32.000	F(2,9)=3.000
	P=0.000	P=0.000	P=0.100

Values are expressed as mean ± SD of four replicates. Mean values within a column with common letter are not significantly different (P = .05) according to LSD test.

4.3.4 Nematicidal effects of *D. stramonium* extracts at 100 % dilution

At 100% concentration, all the extracts resulted in 100% mortality of J2s after 24 hours of exposure. The findings suggests that, type of extract, concentration of the extracts and period of exposure had significant effect on mortality of J2s even after adjusting for natural mortality.

4.3.5 Comparison of effectiveness of seed, root and leaf extracts on mortality of second stage juveniles (J2s) of *G. rostochiensis*

A two-way analysis of variance (ANOVA) conducted indicated that the three extracts were significantly different in their effect on mortality of J2s ($F(2, 45) = 111.032$, $p=0.000$) (Figure 7). Among the three extracts, there was a significant difference in mortality of J2s observed after 24 and 48 hours of exposure to the extracts ($p= 0.000$ and $p= 0.001$ respectively). However after 72 hours, the three extracts did not have any significant difference ($p= 0.204$ and $F= 1.909$). The tests further showed significant difference in the effect of various dilution levels on the mortality of J2s ($F(4, 45) = 888.439$, $p=0.000$). The crude extracts from all parts (roots, leaves and seeds) had the best performance causing 100% mortality within 24h of exposure. In addition, there was significant interaction between type of extract and the level of dilution with respect to their effect on the mortality of J2s ($F(16, 90) = 14.540$, $p=0.000$). The study established that the effect of the various extracts and levels of dilution on mortality of J2s varied with the period of exposure. During the 24 hour period, the three extracts had significantly different effect on the mortality of J2s ($F(2, 11) = 27.940$, $p=0.000$.

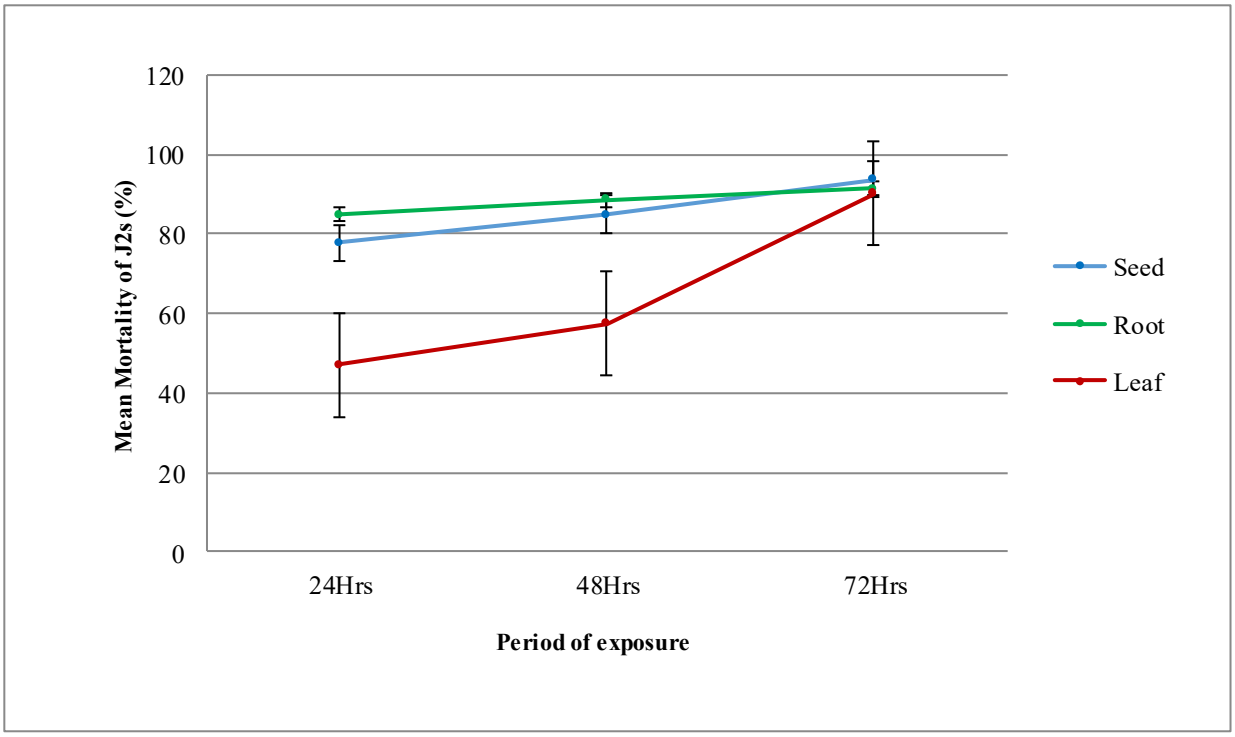

Figure 7: Comparison of the effectiveness of seed, root and leaf extracts on mortality of *G. rostochiensis* J2s.

CHAPTER FIVE
DISCUSSION

5.1 Screening potato varieties for resistance against *Globodera rostochiensis*

This study has established that the six tested potato varieties currently grown by Kenyan potato growers, that is, Shangi, Tigoni, Dutch Robjin, Kenya Mpya, Kenya Karibu and Sherekea, are susceptible to *G. rostochiensis*. However, the levels of susceptibility differed among the varieties with the Reproductive Factor (RF) ranging from 3.85 to 8.80 and the Relative Susceptibility (RS) being 2 and 3. The standard control variety (Desiree) had the highest cyst population which was significantly different from all the tested varieties.

Screening of potato varieties for resistance to *G. rostochiensis* is usually carried out with an aim to identify the varieties that might be having nematode resistance genes (Whitworth *et al.,* 2018). This precedes the long- term process of breeding new potato varieties resistant to the nematode. The current study agrees with other studies carried out earlier that established that some potato varieties are resistant to *G. rostochiensis* while others are susceptible (Faggian *et al.,* 2012) and also that the level of susceptibility differs among the varieties (Renco, 2007). During the study by Faggian and co-workers, 302 varieties were screened for resistance out of which 89 were found to be resistant to *G. rostochiensis* and the rest 213 were susceptible.

Considering the high yield losses associated with *G. rostochiensis* infestation in an area, coupled with the restrictions in international trade and now lack of a popularly grown potato variety that is resistant to the nematode, these are indications of a major setback in potato production in Kenya. Shangi, is one of the most popular variety among the Kenyan potato farmers (MoALF, 2016; NPCK, 2019). It had a significantly lower population of cysts (96.25 cysts) as compared to Kenya Karibu (182.65 cysts) and Sherekea (220 cysts) at $P = 0.000$ and $P = 0.004$ respectively. However the population of *G. rostochiensis* in Shangi was not significantly different with Dutch Robjin ($p = 0.858$). Although the population in Shangi at 96.25 ±37.05 cysts per 1000g of soil was the lowest when compared to the other tested varieties, it was high enough to cause significant loss in yield. The Kenya potato variety catalogue (NPCK, 2019) did not have the information on the susceptibility of the potato varieties tested in this study. However, there are some imported potato varieties that are registered by the NPCK as resistant to the PCN in the country but they are not popularly grown by small scale producers. The relevant authorities should create awareness on the PCN resistant varieties to the farmers to reduce the multiplication of the nematode. This means that presently

the nematode is a serious threat to the quantity of potato produced in the country which translates to reduced food security. Therefore management of nematode population to ensure decline in the affected areas is important.

5.2 Effects of chicken manure on second stage juveniles (J2s) of *G. rostochiensis*

When chicken manure was applied as soil amendment in pots growing the susceptible standard control, the cyst population of *G. rostochiensis* was significantly reduced. A mean difference of 145 cysts was obtained between the amended and unamended soils. The Reproductive Factor (RF) of the potatoes grown on the amended soil was much lower than that of four out of the six potato varieties screened for *G. rostochiensis* resistance. If the population reduction percentage (51.6%) as a result of application of the chicken manure in this study can be replicated at the farm level, then the performance of the best two tested potato varieties (Shangi and Tigoni) can be significantly improved due to low cyst population.

Previous study on effects of chicken manure on J2s of *Meloidogyne incognita* attacking eggplants showed a significant reduction of 81.2 % in J2 population (Osman *et al.,* 2018). Also in another study, the results confirm that compost manure can reduce the reproduction of the potato cyst nematodes to a certain extent (Renco *et al.,* 2011). Poultry manure applied in carrot fields to manage *Meloidogyne* spp reduced root galling and the population of the nematodes significantly (Kankam *et al.,* 2015). The reduction might possibly be due to the Ammonia released during the breakdown of poultry manure in the soil (Amanullah *et al.,* 2010; Chen *et al.,* 2017). Poultry litter contains uric acid, which is converted to urea by the enzyme uricase. Urea is in turn hydrolyzed to ammoniacal N by the enzyme urease, with the process consuming H^+ ions and raising pH (Doydora *et al.,* 2011). Volatilization of NH_3 is a pH-dependent process, with conversion of aqueous NH_4^+ into NH_3 starting around pH 7. The mode of action is thought to be based on the release of toxic levels of ammonium (Abdel- Dayem *et al.,* 2012).

Further, the poultry manure is also known to have some positive effects when applied on the soil. This includes altering the soil structure and stimulating the action of antagonistic organisms that leads to the increase of plant tolerance (Koenning & Barker, 2004; Meyer *et al.,* 2011). The effects play an important role in improving the resistance of plants to pests and diseases which could have led to lower cyst population. This study did not look into the effects of different quantities of chicken manure on *G. rostochiensis* population. Therefore further evaluation can be carried out to find out the most suitable amount of manure that can reduce

the cyst population and maintain high potato yields. The quantity of the manure can also be a factor that can influence the number of cysts reproduced. The reduction in the cyst population in this study indicates that chicken manure can be used in potato farming to manage the nematodes to a great extent.

5.3 Effects of aqueous extracts of *D. stramonium* against second stage juveniles (J2s) of *G. rostochiensis*

This study has established that the extracts of leaves, seeds and roots of *D. stramonium* have nematicidal effects on the J2s of *G. rostochiensis*. The mortality effect of all the extracts varied at the different levels of dilutions, with the highest mortality recorded in the crude extract and the lowest in the control. This is in agreement with a study by Umar and Ngwamdai (2015), where the crude extract of *D. stramonium* recorded the highest mortality (97%) on *Meloidogyne javanica* juveniles followed by 50% dilution (92%) and the least was control at 0%. Adomako and Kwoseh (2013) reported that the crude extract of castor beans caused the highest mortality of *Meloidogyne spp.* juveniles as compared to the lower dilutions.

The J2s mortality also increased with increase in time of exposure. Similarly, in a study by Chaudhary *et al.* (2013, *D. stramonium* was reported to cause mortality on *Meloidogyne incognita* J2s. The mortality caused by the extracts was at 71.5 ± 1.4 % at 50 mg/ ml and 88.7 ± 2.6 % at 100 mg/ ml after 24h exposure. A mortality of 100 % was recorded after 72h exposure at 50 mg/ ml and 100 mg/ ml. The work carried out previously by Oplos *et al.* (2018) also show that time of exposure to *D. stramonium* extracts affected the mortality of *Meloidogyne javanica* and *Meloidogyne incognita*. The mortality of J2s in the present study can possibly be due to the presence of metabolites in *D. stramonium,* such as alkaloids, saponins, phenols, steroids flavonoids and tannins (Girmay, 2015; Sayyed & Shah, 2014). While this study does not identify the active components in the extracts that caused the J2s mortality, the metabolites have been reported to have negative effects on the body systems of organisms. Alkaloids such as Atropine, Hyoscyamine and Scopolamine affect the nervous system with Atropines depressing the smooth muscles and secretory glands (Carpa *et al.,* 2017). The Saponins have been reported to interact with the collagen protein in the cuticle of nematodes (Argentieri *et al.*, 2008). They also cause changes in the permeability of the cell membrane which leads to the mortality of the J2s (Ibrahim & Srour, 2013). Similarly, Adegbite and Adesiyan (2005) reported that the presence of tannins, alkaloids and flavonoids in *D. stramonium* leaves are known to kill nematodes. The complexity of the biological interactions

among the chemical constituents of the plant may also be contributing to synergistic performance of the extracts.

The performance of the three extracts evaluated on the mortality of the *G. rostochiensis* J2s was significantly different in 24 and 48h. The root extract caused the highest mortality, followed by the seed and lastly the leaf extract. Interestingly, the performance of the leaf extract increased exponentially between 48 and 72h of exposure. Without bio- chemical analysis of the extracts, there are limitations in identifying the specific chemicals involved in the J2 mortality. The difference in performance however, can be attributed to the difference in the levels of the metabolites in different plant parts at different times of their growth (Iranbakhsh *et al.,* 2006). The leaves contain slight amounts of Alkaloids, moderate amounts of Tannins and a high amount of Saponins and Flavonoids (Umar & Ngwamdai, 2015). The seeds have many amino acids and high levels of Atropines while the roots have low levels of Scopolamine and moderate amounts of Atropines (Al- Snafi, 2017).

Since *D. stramonium* is a widespread weed in Kenya and its parts have the ability to reduce the population of J2s *in vitro,* it can be tested as an alternative tool to control the nematodes. In Kenya, organic nematicides such as Carbofuran are regulated due to their high toxicity. Hence this plant can be exploited as a potential eco- friendly bionematicide against *G. rostochiensis* in potatoes. This would be an alternative to the hazardous synthetic nematicides. There is need to carry out further studies to identify the mode of action of the phytochemicals present in *D. stramonium* against the PCN. This may lead to development of new and low cost nematicidal and nematistatic formulations from this plant.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were made from the study;

i) All the test varieties in this study were susceptible to *G. rostochiensis* but the level of susceptibility differed. Shangi and Tigoni had the lowest susceptibility while Kenya Karibu and Sherekea had the highest susceptibility.

ii) Chicken manure has the ability to reduce the population of the potato cyst nematodes significantly. It can therefore be used to plant potatoes by especially the small scale producers to manage the nematode in their farms.

iii) This study established that the leaf, seed and root extracts of *D. stramonium* had nematicidal effects on the J2s of *G. rostochiensis in vitro.* The type of extract, level of dilution and the period of exposure affected the mortality rate of the J2s.

6.2 Recommendations

The following are the recommendations from the study;

i) The farmers should be made aware of the susceptibility of the tested varieties so as to make informed choices on the variety to grow.

ii) Other varieties that are known to be resistant should be recommended for production.

iii) Chicken manure should be included in the production of potatoes by the small scale farmers to manage the PCN.

iv) Further studies should be done to establish the rate of application of the chicken manure per area to avoid over or under fertilization of the farms.

v) There is need to carry out further studies to identify the metabolites present in the extracts, their mode of action and the level of application for effective management of *G. rostochiensis.*

vi) These extracts are recommended for development of new and low cost nematicidal and nematistatic formulations against *G. rostochiensis.*

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APPENDICES

Appendix 1: Analysis of the performance of test varieties screened for resistance against *G. rostochiensis*

Means of *G. rostochiensis* cyst population for the different test varieties

Test Variety	N	Mean	Std. Deviation	Std. Error
Shangi	4	96.2500	37.05289	18.52645
Dutch Robjin	4	161.2500	32.75541	16.37770
Tigoni	4	101.2500	31.19161	15.59581
Kenya Mpya	4	162.5000	33.78856	16.89428
Kenya Karibu	4	220.0000	44.90731	22.45366
Sherekea	4	186.2500	50.22864	25.11432
Total	24	154.5833	56.66294	11.56627

ANOVA table

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46545.833	5	9309.167	6.138	.002
Within Groups	27300.000	18	1516.667		
Total	73845.833	23			

Separation of cyst population means among the test varieties

	(I) Potato variety	(J) Potato variety	Mean Difference (I-J)	Std. Error	Sig.
LSD	Shangi	Dutch Robjin	-65.00000*	27.53785	.030
		Tigoni	-5.00000	27.53785	.858
		Kenya Mpya	-66.25000*	27.53785	.027
		Kenya Karibu	-123.75000*	27.53785	.000
		Sherekea	-90.00000*	27.53785	.004
	Dutch Robjin	Shangi	65.00000*	27.53785	.030
		Tigoni	60.00000*	27.53785	.043
		Kenya Mpya	-1.25000	27.53785	.964
		Kenya Karibu	-58.75000*	27.53785	.047
		Sherekea	-25.00000	27.53785	.376
	Tigoni	Shangi	5.00000	27.53785	.858
		Dutch Robjin	-60.00000*	27.53785	.043
		Kenya Mpya	-61.25000*	27.53785	.039
		Kenya Karibu	-118.75000*	27.53785	.000
		Sherekea	-85.00000*	27.53785	.006
	Kenya Mpya	Shangi	66.25000*	27.53785	.027
		Dutch Robjin	1.25000	27.53785	.964
		Tigoni	61.25000*	27.53785	.039
		Kenya Karibu	-57.50000	27.53785	.051
		Sherekea	-23.75000	27.53785	.400
	Kenya Karibu	Shangi	123.75000*	27.53785	.000
		Dutch Robjin	58.75000*	27.53785	.047
		Tigoni	118.75000*	27.53785	.000
		Kenya Mpya	57.50000	27.53785	.051
		Sherekea	33.75000	27.53785	.236
	Sherekea	Shangi	90.00000*	27.53785	.004
		Dutch Robjin	25.00000	27.53785	.376
		Tigoni	85.00000*	27.53785	.006
		Kenya Mpya	23.75000	27.53785	.400
		Kenya Karibu	-33.75000	27.53785	.236

*. The mean difference is significant at the 0.05 level.

Appendix 2: Analysis of the effects of individual extracts of *D. stramonium* on the mortality of J2s

Mean mortality of seed extract at different dilutions for day 1, 2 and 3.

		N	Mean	Std. Deviation	Std. Error
Day1	DW	4	2.5000	2.88675	1.44338
	12.5%	4	31.2500	8.53913	4.26956
	25%	4	82.5000	8.66025	4.33013
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	63.2500	40.79070	9.12108
Day2	DW	4	12.5000	5.00000	2.50000
	12.5%	4	57.5000	11.90238	5.95119
	25%	4	90.0000	7.07107	3.53553
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	72.0000	34.95862	7.81699
Day3	DW	4	30.0000	7.07107	3.53553
	12.5%	4	87.5000	6.45497	3.22749
	25%	4	95.0000	7.07107	3.53553
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	82.5000	27.74413	6.20378

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day 1	Between Groups	31145.000	4	7786.250	249.160	.000
	Within Groups	468.750	15	31.250		
	Total	31613.750	19			
Day 2	Between Groups	22570.000	4	5642.500	130.212	.000
	Within Groups	650.000	15	43.333		
	Total	23220.000	19			
Day 3	Between Groups	14200.000	4	3550.000	125.294	.000
	Within Groups	425.000	15	28.333		
	Total	14625.000	19			

Separation of means for J2 mortality (%) for seed extract at different dilutions for day 1, 2 and 3.

Dependent Variable		(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	DW	12.5%	-28.75000*	3.95285	.000
			25%	-80.00000*	3.95285	.000
			50%	-97.50000*	3.95285	.000
			100%	-97.50000*	3.95285	.000
		12.5%	DW	28.75000*	3.95285	.000
			25%	-51.25000*	3.95285	.000
			50%	-68.75000*	3.95285	.000
			100%	-68.75000*	3.95285	.000
		25%	DW	80.00000*	3.95285	.000
			12.5%	51.25000*	3.95285	.000
			50%	-17.50000*	3.95285	.000
			100%	-17.50000*	3.95285	.000
		50%	DW	97.50000*	3.95285	.000
			12.5%	68.75000*	3.95285	.000
			25%	17.50000*	3.95285	.000
			100%	.00000	3.95285	1.000
		100%	DW	97.50000*	3.95285	.000
			12.5%	68.75000*	3.95285	.000
			25%	17.50000*	3.95285	.000
			50%	.00000	3.95285	1.000
Day 2	LSD	DW	12.5%	-45.00000*	4.65475	.000
			25%	-77.50000*	4.65475	.000
			50%	-87.50000*	4.65475	.000
			100%	-87.50000*	4.65475	.000
		12.5%	DW	45.00000*	4.65475	.000
			25%	-32.50000*	4.65475	.000
			50%	-42.50000*	4.65475	.000
			100%	-42.50000*	4.65475	.000

		25%	DW	77.50000*	4.65475	.000
			12.5%	32.50000*	4.65475	.000
			50%	-10.00000*	4.65475	.048
			100%	-10.00000*	4.65475	.048
		50%	DW	87.50000*	4.65475	.000
			12.5%	42.50000*	4.65475	.000
			25%	10.00000*	4.65475	.048
			100%	.00000	4.65475	1.000
		100%	DW	87.50000*	4.65475	.000
			12.5%	42.50000*	4.65475	.000
			25%	10.00000*	4.65475	.048
			50%	.00000	4.65475	1.000
Day 3	LSD	DW	12.5%	-57.50000*	3.76386	.000
			25%	-65.00000*	3.76386	.000
			50%	-70.00000*	3.76386	.000
			100%	-70.00000*	3.76386	.000
		12.5%	DW	57.50000*	3.76386	.000
			25%	-7.50000	3.76386	.065
			50%	-12.50000*	3.76386	.005
			100%	-12.50000*	3.76386	.005
		25%	DW	65.00000*	3.76386	.000
			12.5%	7.50000	3.76386	.065
			50%	-5.00000	3.76386	.204
			100%	-5.00000	3.76386	.204
		50%	DW	70.00000*	3.76386	.000
			12.5%	12.50000*	3.76386	.005
			25%	5.00000	3.76386	.204
			100%	.00000	3.76386	1.000
		100%	DW	70.00000*	3.76386	.000
			12.5%	12.50000*	3.76386	.005
			25%	5.00000	3.76386	.204
			50%	.00000	3.76386	1.000

* The mean difference is significant at the 0.05 level.

Mean mortality of root extract at different dilutions for day 1, 2 and 3.

		N	Mean	Std. Deviation	Std. Error
Day1	DW	4	2.5000	2.88675	1.44338
	12.5%	4	65.0000	12.90994	6.45497
	25%	4	76.2500	7.50000	3.75000
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	68.7500	37.23591	8.32620
Day2	DW	4	12.5000	5.00000	2.50000
	12.5%	4	75.0000	7.07107	3.53553
	25%	4	85.0000	9.12871	4.56435
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	74.5000	33.63504	7.52102
Day3	DW	4	30.0000	7.07107	3.53553
	12.5%	4	80.0000	4.08248	2.04124
	25%	4	96.2500	7.50000	3.75000
	50%	4	100.0000	.00000	.00000
	100%	4	100.0000	.00000	.00000
	Total	20	81.2500	27.71447	6.19714

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day 1	Between Groups	25650.000	4	6412.500	138.649	.000
	Within Groups	693.750	15	46.250		
	Total	26343.750	19			
Day 2	Between Groups	21020.000	4	5255.000	165.947	.000
	Within Groups	475.000	15	31.667		
	Total	21495.000	19			
Day 3	Between Groups	14225.000	4	3556.250	144.661	.000
	Within Groups	368.750	15	24.583		
	Total	14593.750	19			

Separation of means of J2 mortality (%) for root extract at different dilution for day 1, 2 and 3

Dependent Variable		(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	DW	12.5%	-62.50000*	4.80885	.000
			25%	-73.75000*	4.80885	.000
			50%	-97.50000*	4.80885	.000
			100%	-97.50000*	4.80885	.000
		12.5%	DW	62.50000*	4.80885	.000
			25%	-11.25000*	4.80885	.034
			50%	-35.00000*	4.80885	.000
			100%	-35.00000*	4.80885	.000
		25%	DW	73.75000*	4.80885	.000
			12.5%	11.25000*	4.80885	.034
			50%	-23.75000*	4.80885	.000
			100%	-23.75000*	4.80885	.000
		50%	DW	97.50000*	4.80885	.000
			12.5%	35.00000*	4.80885	.000
			25%	23.75000*	4.80885	.000
			100%	.00000	4.80885	1.000
		100%	DW	97.50000*	4.80885	.000
			12.5%	35.00000*	4.80885	.000
			25%	23.75000*	4.80885	.000
			50%	.00000	4.80885	1.000
Day 2	LSD	DW	12.5%	-62.50000*	3.97911	.000
			25%	-72.50000*	3.97911	.000
			50%	-87.50000*	3.97911	.000
			100%	-87.50000*	3.97911	.000
		12.5%	DW	62.50000*	3.97911	.000
			25%	-10.00000*	3.97911	.024
			50%	-25.00000*	3.97911	.000
			100%	-25.00000*	3.97911	.000

		25%	DW	72.50000*	3.97911	.000
			12.5%	10.00000*	3.97911	.024
			50%	-15.00000*	3.97911	.002
			100%	-15.00000*	3.97911	.002
		50%	DW	87.50000*	3.97911	.000
			12.5%	25.00000*	3.97911	.000
			25%	15.00000*	3.97911	.002
			100%	.00000	3.97911	1.000
		100%	DW	87.50000*	3.97911	.000
			12.5%	25.00000*	3.97911	.000
			25%	15.00000*	3.97911	.002
			50%	.00000	3.97911	1.000
Day 3	LSD	DW	12.5%	-50.00000*	3.50595	.000
			25%	-66.25000*	3.50595	.000
			50%	-70.00000*	3.50595	.000
			100%	-70.00000*	3.50595	.000
		12.5%	DW	50.00000*	3.50595	.000
			25%	-16.25000*	3.50595	.000
			50%	-20.00000*	3.50595	.000
			100%	-20.00000*	3.50595	.000
		25%	DW	66.25000*	3.50595	.000
			12.5%	16.25000*	3.50595	.000
			50%	-3.75000	3.50595	.302
			100%	-3.75000	3.50595	.302
		50%	DW	70.00000*	3.50595	.000
			12.5%	20.00000*	3.50595	.000
			25%	3.75000	3.50595	.302
			100%	.00000	3.50595	1.000
		100%	DW	70.00000*	3.50595	.000
			12.5%	20.00000*	3.50595	.000
			25%	3.75000	3.50595	.302
			50%	.00000	3.50595	1.000

* The mean difference is significant at the 0.05 level.

Mean mortality of leaf extract at different dilutions for day 1, 2 and 3.

		N	Mean	Std. Deviation	Std. Error
Day1	DW	4	2.5000	2.88675	1.44338
	12.5%	4	18.7500	2.50000	1.25000
	25%	4	31.2500	12.50000	6.25000
	50%	4	43.7500	4.78714	2.39357
	100%	4	100.0000	.00000	.00000
	Total	20	39.2500	34.61347	7.73981
Day2	DW	4	12.5000	5.00000	2.50000
	12.5%	4	33.7500	8.53913	4.26956
	25%	4	37.5000	8.66025	4.33013
	50%	4	80.0000	7.07107	3.53553
	100%	4	100.0000	.00000	.00000
	Total	20	52.7500	33.57925	7.50855
Day3	DW	4	30.0000	7.07107	3.53553
	12.5%	4	85.0000	5.77350	2.88675
	25%	4	90.0000	4.08248	2.04124
	50%	4	97.5000	2.88675	1.44338
	100%	4	100.0000	.00000	.00000
	Total	20	80.5000	26.79847	5.99232

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day1	Between Groups	22182.500	4	5545.625	143.113	.000
	Within Groups	581.250	15	38.750		
	Total	22763.750	19			
Day2	Between Groups	20755.000	4	5188.750	116.383	.000
	Within Groups	668.750	15	44.583		
	Total	21423.750	19			
Day3	Between Groups	13320.000	4	3330.000	153.692	.000
	Within Groups	325.000	15	21.667		
	Total	13645.000	19			

Separation of means for J2 mortality (%) for leaf extract at different dilutions for day 1, 2 and 3.

Dependent Variable		(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	DW	12.5%	-16.25000*	4.40170	.002
			25%	-28.75000*	4.40170	.000
			50%	-41.25000*	4.40170	.000
			100%	-97.50000*	4.40170	.000
		12.5%	DW	16.25000*	4.40170	.002
			25%	-12.50000*	4.40170	.012
			50%	-25.00000*	4.40170	.000
			100%	-81.25000*	4.40170	.000
		25%	DW	28.75000*	4.40170	.000
			12.5%	12.50000*	4.40170	.012
			50%	-12.50000*	4.40170	.012
			100%	-68.75000*	4.40170	.000
		50%	DW	41.25000*	4.40170	.000
			12.5%	25.00000*	4.40170	.000
			25%	12.50000*	4.40170	.012
			100%	-56.25000*	4.40170	.000
		100%	DW	97.50000*	4.40170	.000
			12.5%	81.25000*	4.40170	.000
			25%	68.75000*	4.40170	.000
			50%	56.25000*	4.40170	.000
Day 2	LSD	DW	12.5%	-21.25000*	4.72141	.000
			25%	-25.00000*	4.72141	.000
			50%	-67.50000*	4.72141	.000
			100%	-87.50000*	4.72141	.000
		12.5%	DW	21.25000*	4.72141	.000
			25%	-3.75000	4.72141	.439
			50%	-46.25000*	4.72141	.000
			100%	-66.25000*	4.72141	.000

		25%	DW	25.00000*	4.72141	.000
			12.5%	3.75000	4.72141	.439
			50%	-42.50000*	4.72141	.000
			100%	-62.50000*	4.72141	.000
		50%	DW	67.50000*	4.72141	.000
			12.5%	46.25000*	4.72141	.000
			25%	42.50000*	4.72141	.000
			100%	-20.00000*	4.72141	.001
		100%	DW	87.50000*	4.72141	.000
			12.5%	66.25000*	4.72141	.000
			25%	62.50000*	4.72141	.000
			50%	20.00000*	4.72141	.001
Day 3	LSD	DW	12.5%	-55.00000*	3.29140	.000
			25%	-60.00000*	3.29140	.000
			50%	-67.50000*	3.29140	.000
			100%	-70.00000*	3.29140	.000
		12.5%	DW	55.00000*	3.29140	.000
			25%	-5.00000	3.29140	.150
			50%	-12.50000*	3.29140	.002
			100%	-15.00000*	3.29140	.000
		25%	DW	60.00000*	3.29140	.000
			12.5%	5.00000	3.29140	.150
			50%	-7.50000*	3.29140	.038
			100%	-10.00000*	3.29140	.008
		50%	DW	67.50000*	3.29140	.000
			12.5%	12.50000*	3.29140	.002
			25%	7.50000*	3.29140	.038
			100%	-2.50000	3.29140	.459
		100%	DW	70.00000*	3.29140	.000
			12.5%	15.00000*	3.29140	.000
			25%	10.00000*	3.29140	.008
			50%	2.50000	3.29140	.459

* The mean difference is significant at the 0.05 level.

Appendix 3: Comparison of effectiveness of seed, root and leaf extracts on J2 mortality at different dilutions within 72hours

Mean mortality (%) of J2s for seed, leaf and root extracts at 12.5% dilution

Time	Extract	N	Mean	Std. Deviation	Std. Error
Day 1	Seed	4	31.2500	8.53913	4.26956
	Root	4	65.0000	12.90994	6.45497
	Leaf	4	18.7500	2.50000	1.25000
	Total	12	38.3333	21.98484	6.34648
Day 2	Seed	4	57.5000	11.90238	5.95119
	Root	4	75.0000	7.07107	3.53553
	Leaf	4	33.7500	8.53913	4.26956
	Total	12	55.4167	19.59340	5.65613
Day 3	Seed	4	87.5000	6.45497	3.22749
	Root	4	80.0000	4.08248	2.04124
	Leaf	4	85.0000	5.77350	2.88675
	Total	12	84.1667	5.96708	1.72255

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day1	Between Groups	4579.167	2	2289.583	27.941	.000
	Within Groups	737.500	9	81.944		
	Total	5316.667	11			
Day2	Between Groups	3429.167	2	1714.583	19.441	.001
	Within Groups	793.750	9	88.194		
	Total	4222.917	11			
Day3	Between Groups	116.667	2	58.333	1.909	.204
	Within Groups	275.000	9	30.556		
	Total	391.667	11			

Separation of means in J2 mortality (%) for seed, leaf and root extracts at 12.5%

Dependent Variable		(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	Seed	Root	-33.75000*	6.40095	.001
			Leaf	12.50000	6.40095	.083
		Root	Seed	33.75000*	6.40095	.001
			Leaf	46.25000*	6.40095	.000
		Leaf	Seed	-12.50000	6.40095	.083
			Root	-46.25000*	6.40095	.000
Day 2	LSD	Seed	Root	-17.50000*	6.64057	.027
			Leaf	23.75000*	6.64057	.006
		Root	Seed	17.50000*	6.64057	.027
			Leaf	41.25000*	6.64057	.000
		Leaf	Seed	-23.75000*	6.64057	.006
			Root	-41.25000*	6.64057	.000
Day 3	LSD	Seed	Root	7.50000	3.90868	.087
			Leaf	2.50000	3.90868	.538
		Root	Seed	-7.50000	3.90868	.087
			Leaf	-5.00000	3.90868	.233
		Leaf	Seed	-2.50000	3.90868	.538
			Root	5.00000	3.90868	.233

* The mean difference is significant at the 0.05 level.

Mean mortality (%) of J2s for seed, leaf and root extracts at 25% dilution

		N	Mean	Std. Deviation	Std. Error
Day1	Seed	4	82.5000	8.66025	4.33013
	Root	4	76.2500	7.50000	3.75000
	Leaf	4	31.2500	12.50000	6.25000
	Total	12	63.3333	25.43560	7.34262
Day2	Seed	4	90.0000	7.07107	3.53553
	Root	4	85.0000	9.12871	4.56435
	Leaf	4	37.5000	8.66025	4.33013
	Total	12	70.8333	25.83456	7.45779
Day3	Seed	4	95.0000	7.07107	3.53553
	Root	4	96.2500	7.50000	3.75000
	Leaf	4	90.0000	4.08248	2.04124
	Total	12	93.7500	6.44029	1.85915

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day 1	Between Groups	6254.167	2	3127.083	32.630	.000
	Within Groups	862.500	9	95.833		
	Total	7116.667	11			
Day 2	Between Groups	6716.667	2	3358.333	48.360	.000
	Within Groups	625.000	9	69.444		
	Total	7341.667	11			
Day 3	Between Groups	87.500	2	43.750	1.068	.384
	Within Groups	368.750	9	40.972		
	Total	456.250	11			

Separation of means in J2 mortality (%) for seed, leaf and root extracts at 25%

Dependent Variable		(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	Seed	Root	6.25000	6.92219	.390
			Leaf	51.25000*	6.92219	.000
		Root	Seed	-6.25000	6.92219	.390
			Leaf	45.00000*	6.92219	.000
		Leaf	Seed	-51.25000*	6.92219	.000
			Root	-45.00000*	6.92219	.000
Day 2	LSD	Seed	Root	5.00000	5.89256	.418
			Leaf	52.50000*	5.89256	.000
		Root	Seed	-5.00000	5.89256	.418
			Leaf	47.50000*	5.89256	.000
		Leaf	Seed	-52.50000*	5.89256	.000
			Root	-47.50000*	5.89256	.000
Day 3	LSD	Seed	Root	-1.25000	4.52616	.789
			Leaf	5.00000	4.52616	.298
		Root	Seed	1.25000	4.52616	.789
			Leaf	6.25000	4.52616	.201
		Leaf	Seed	-5.00000	4.52616	.298
			Root	-6.25000	4.52616	.201

* The mean difference is significant at the 0.05 level.

Mean mortality (%) of J2s for seed, leaf and root extracts at 50% dilution

		N	Mean	Std. Deviation	Std. Error
Day1	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	43.7500	4.78714	2.39357
	Total	12	81.2500	27.80819	8.02753
Day2	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	80.0000	7.07107	3.53553
	Total	12	93.3333	10.51694	3.03598
Day3	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	97.5000	2.88675	1.44338
	Total	12	99.1667	1.94625	.56183

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day 1	Between Groups	8437.500	2	4218.750	552.273	.000
	Within Groups	68.750	9	7.639		
	Total	8506.250	11			
Day 2	Between Groups	1066.667	2	533.333	32.000	.000
	Within Groups	150.000	9	16.667		
	Total	1216.667	11			
Day 3	Between Groups	16.667	2	8.333	3.000	.100
	Within Groups	25.000	9	2.778		
	Total	41.667	11			

Separation of means in J2 mortality (%) for seed, leaf and root extracts at 50%

Dependent Variable		(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.
Day 1	LSD	Seed	Root	.00000	1.95434	1.000
			Leaf	56.25000*	1.95434	.000
		Root	Seed	.00000	1.95434	1.000
			Leaf	56.25000*	1.95434	.000
		Leaf	Seed	-56.25000*	1.95434	.000
			Root	-56.25000*	1.95434	.000
Day 2	LSD	Seed	Root	.00000	2.88675	1.000
			Leaf	20.00000*	2.88675	.000
		Root	Seed	.00000	2.88675	1.000
			Leaf	20.00000*	2.88675	.000
		Leaf	Seed	-20.00000*	2.88675	.000
			Root	-20.00000*	2.88675	.000
Day 3	LSD	Seed	Root	.00000	1.17851	1.000
			Leaf	2.50000	1.17851	.063
		Root	Seed	.00000	1.17851	1.000
			Leaf	2.50000	1.17851	.063
		Leaf	Seed	-2.50000	1.17851	.063
			Root	-2.50000	1.17851	.063

* The mean difference is significant at the 0.05 level.

Mean mortality (%) of J2s for seed, leaf and root extracts at 100% dilution

		N	Mean	Std. Deviation	Std. Error
Day1	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	100.0000	.00000	.00000
	Total	12	100.0000	.00000	.00000
Day2	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	100.0000	.00000	.00000
	Total	12	100.0000	.00000	.00000
Day3	Seed	4	100.0000	.00000	.00000
	Root	4	100.0000	.00000	.00000
	Leaf	4	100.0000	.00000	.00000
	Total	12	100.0000	.00000	.00000

ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
Day 1	Between Groups	.000	2	.000	.	.
	Within Groups	.000	9	.000		
	Total	.000	11			
Day 2	Between Groups	.000	2	.000	.	.
	Within Groups	.000	9	.000		
	Total	.000	11			
Day 3	Between Groups	.000	2	.000	.	.
	Within Groups	.000	9	.000		
	Total	.000	11			

Appendix 4: Abstract page of the published paper

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Research Paper
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Effect of aqueous extracts of *Datura stramonium* W. seeds, leaves and roots on mortality of second stage juveniles of *Globodera rostochiensis* in Kenya

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Abstract

Experiments were carried out in the laboratory to evaluate the effects of aqueous extracts of leaves, seeds and roots of *Datura stramonium* at different concentrations on mortality of *Globodera rostochiensis* juveniles. Volumes of 5 mL of the extract at 100 (crude extract), 50, 25 and 12.5% dilutions were dispensed separately into Petri dishes containing 20 freshly hatched second stage juveniles (J2s) of *G. rostochiensis*. Juvenile mortality was recorded at 24, 48 and 72 h intervals. The treatments were replicated four times. Petri dishes were arranged in a Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was used to test for the differences (means) among the extracts and the dilutions. The results showed that the crude extracts (100%) of all extracts and 50% dilution of roots and seeds caused 100% mortality after 24 h exposure. This study established that aqueous extracts of *D. stramonium* has the potential for use as a bionematicide. Further experiments are required to confirm their effect under cropping conditions. There is also a need to analyze the phytochemicals of the plant which cause nematode mortality.

Keywords: *Datura stramonium, Globodera rostochiensis, Nematode mortality, Phytochemicals, Second stage juveniles (J2s)*

1. Introduction

Plant Parasitic Nematodes (PPN) feed on roots and complete their life cycles in or on the root zone and in a few cases in the shoot. They affect both the quality and quantity of marketable yields of agricultural crops (Daramola et al., 2015). The *Globodera rostochiensis* also referred to as yellow/golden potato cyst, is a Potato Cyst Nematode (PCN) that causes serious yield loss in infested potato growing areas and can cause up to 80% yield loss under heavy infestation (CABI, 2019). The pest is also difficult to eradicate once it infests an area since the eggs are encased in cysts that can remain viable for many years in the soil without a host (Coyne et al., 2018). This poses a serious threat to potato production where the nematode is present. The PCN is a quarantine pest in most

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Appendix 5: Research permit

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