

**MANAGEMENT OF ROOT-KNOT NEMATODES IN CARROTS
USING NEEM (*AZADIRACTHA INDICA*) LEAF EXTRACT**

BY

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DECLARATION

I, Namiwanda Ruth, declare that this my original work and has never been submitted to any other institution of higher learning for any award. Scholarly work of others was cited and acknowledged throughout the entire text.

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APPROVAL

This research report titled “Management of root-knot nematodes in carrots using neem leaf extract (*Azadiractha indica*)” was carried out under my guidance and has been cleared for submission to Kyambogo University for examination.

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DEDICATION

I dedicate this dissertation to my family for the support extended to me during my education.

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I extend my heartfelt gratitude to the merciful and Almighty God for his endless blessings, knowledge, wisdom, good health given to me for successful completion of this research study.

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ABSTRACT

The study was conducted to assess the effect the neem tree leaves (*Azadirachta indica*) extract on the management of root-knot nematodes in carrots. The study specifically determined the effect of *A. indica* leaf extract concentration in management of root-knot nematodes in carrots; and determined the effect of *A. indica* leaf extract concentration that suppresses galling and forking incidence on carrot tubers. The experiments were conducted at the Kyambogo University farm in two rainfall seasons of 2019B and 2020A. Treatments composed of three levels of concentration of *A. indica* leaf extract (i.e 15, 25 and 35g) plus a positive control (distilled water) and a negative control (no inoculum). Data was collected included number of standing leaves on the plant, height of the shoot, fresh and dry weight of leaves, fresh weight of carrot tubers and gall incidence. Results showed that there was no significant difference between the negative control (no inoculum applied), distilled water (inoculum applied) and highest concentration level of neem leaf extract (35g) on the number of standing leaves in carrots. Neem leaf extract was found to have significant effect on galling and forking incidence of carrot tubers ($P < 0.001$) The study provides evidence of the effectiveness of neem tree leaves extracts in the management of root- knot nematodes in carrots.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Carrot (*Daucus carota*) is a root vegetable belonging to Apiacea family and its origin is in the Asia. Carrot production has been constrained worldwide by plant-parasitic nematodes (*Meloidogyne* spp) (Viric *et al.*, 2022). The developing countries especially in the Asia and subsaharan Africa are under threat as 60% of the carrot plantations are heavily infestated with *Meloidogyne* spp (Baptista *et al.*, 2022). Uganda is one of the hardest hit countries in sub saharan Africa as 73% of the carrot plantations are attacked by *Meloidogyne* spp. (Isabirye, 2016). This nematode effect compromises zero hunger and improved wel being among Ugandans since, carrot yield has encounted 55.1% yield loss (Seenivasan, 2017).

The farmers still continue to confuse nematodes effect with other biotic and abiotic signs and symptoms like distorted and curled leaves to be caused by *Alternaria* spp; while reduced root length, fibrous roots, poor root colour, and cracking of root tubers to be due to variation in temperature and turgor pressure (Mondal *et al.*, 2020). Carrots have important nutritional attributes such as vitamin A for improving eye sight, it protects the body against prostate cancer and leukaemia due to its carotene composition. It also protects the body against heart disease, sun damage and aging with its antioxidants. Furthermore, it stimulates health of the gum, and scrapes plaque off the teeth (Nakitto, 2017).

There have been efforts to manage root-knot nematodes majorly by use of nematicides which have proved to be very successful. But agro-chemicals, have hazardous effect on the environment as they contain organophosphates, carbamates

and methylbromide that take long to degrade into soil, and hence toxic to non-target organisms. Resistance to agro-chemicals may also develop in nematodes (Ismail, 2020). A number of plant species have been recognized for their nematocidal effects on root knot worms for example, various neem sections have been shown to contain over 40 terpenoids with bitter characteristics, including *azadirachtin*, *salannin*, *nimbin*, *thionemon*, and *nimbidin* (Azlay, 2023).

The Neem tree is reported to have originated from India and Myanmar (Sujarwo *et al.*, 2016). However, it has now spread extensively in most climates of tropical and subtropical countries. *Azadirachtin* component which is an effective pesticide attracting attention worldwide occurs in different parts of the tree, namely, seeds, leaves and the tree bark. *Azadirachtin*, when used as nematodecides act in many ways such as being toxic, prevent the feeding action, regulate the growth of nematodes, repel of oviposition and cuticle damage in larva preventing moulting (Sharma, 2016). It has been demonstrated by Devi *et al.*, (2020) that neem products can reduce phytoparasitic nematodes and improve plant healthy.

Given the negative effects of using nematicides as outlined above, more efforts should be directed to developing organic pesticides and manage all the destructive stages of nematodes. This in one way or the other will improve the quality and quantity of carrot plants (Szelag *et al.*, 2019). Organic pesticides have been reported to manage nematode species such as *Melodigyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Jang, 2019).

1.2 Statement of the problem

Carrot is one of the most important root vegetables grown and produced worldwide. It is a high value crop, rich in vitamin A that promotes good vision in humans (Nakitto, 2017). It is quick maturing and high yielding under optimum growth

conditions as this boosts the livelihood of poor smallholder farmers cultivating it. Important as it is, the quality and yield of the crop is highly affected by both abiotic and biotic factors especially root-knot nematodes accounting for 55.1% yield loss (Seenivasan, 2017). These nematodes are very difficult to manage due to their microscopic nature, confusing symptoms they incite and underground habitat (Teixeira & Kaloshian, 2018). Root-knot nematodes have been eliminated with nematicides, which have been advised and applied successfully. However, the effect of continuous use of nematicides has proved counterproductive on the environment. The majority of these nematicides contain organophosphates, carbamates and methylbromide which take long to degrade. Many studies have been carried out to engage other means of reducing nematode population other than using nematicides, with the view of improving the quality and yield. Since they are toxic to nematodes and disrupt their life cycle stages, which improves plant growth and yield, organic biodegradable pesticides like *Azadirachtin*, which is derived from neem leaf extracts, are effective in controlling root-knot nematodes (*Meloidogyne spp.*) in carrots (Errico *et al.*, 2023).

However, little is known how *Azadirachtin* could control root-knot nematodes in carrots. Yet the use of *Azadirachtin* has already been carried out in crops such as sweet gourd with great success (Mbatyoti *et al.*, 2018) and in Okra (Resha & Savita, 2015). Therefore, it was from this observation that the study was carried out to investigate effect of neem tree leaves in controlling root-knot nematodes of carrots.

1.3 Objectives

1.3.1 Main objective

The general objective of the study was to assess the potential of neem (*Azadirachta indica*) tree leaf extracts in the management of root- knot nematodes in carrots.

1.3.2 Specific objectives

- i. To determine the effect of *Azadirachta indica* leaf extract concentration on growth and yield of carrots
- ii. To determine the effect of *Azadirachta indica* leaf extract concentration on galling and forking incidence of carrot tubers

1.3.3 Research hypothesis

- i. *Azadirachta indica* leaf extract concentration has an effect on growth and yield of carrots
- ii. *Azadirachta indica* leaf extract concentration suppresses galling and forking incidence of carrot tubers

1.4 Significance of the study

The findings of this study will be used for efficient and effective management of root knot nematodes in carrots. It will therefore, improve the quality and yield of carrot tubers, and hence increasing their availability on market. This will go a long way to improve the deficient level of vitamin A among farmers and other consumers.

The findings of this study are to help the key stakeholders on carrot production in Uganda and farmers in other parts of the country in selecting the right concentration level of *A. indica* leaf extract to control root-knot nematodes.

1.5 Scope of the study

The research was carried out to assess the effectiveness of using neem tree leaf extract to control root-knot nematodes associated with carrots at Kyambogo university farm, Uganda from 2019-2021. The treatments in the study included three levels 15g, 25g and 35g of neem tree leaf extract, the positive and negative control respectively. The experiments were conducted in a completely randomised design

and the treatments replicated five times. The dependent variables included number of standing leaves of carrots, weight of fresh leaves, weight of dry leaves, height of the shoot, length of carrot tubers, girth of carrot tuber, weight of carrot tubers, gall and forking incidence on carrot tubers to determine efficacy of *A. indica* leaf extracts of different concentration.

CHAPTER TWO: LITERATURE REVIEW

2.1 Carrot and its importance

In most temperate regions of the world, carrots are regarded as a more significant vegetable root crop. Over the past 40 years, there has been a significant growth in the consumption of carrots (Simon, 2021). Eastern and western carrots are the two main cultures that make up the carrots. Based on their colour, eastern carrots fall into this category as purple and yellow and western carrots fall into this category as orange, red or white. The most popular and extensively cultivated cultivar worldwide is orange (Tyler & Zhongli, 2019).

The carrot was introduced in Punjab or Kashmir in middle Asia and then transferred to the Near East, Europe, and China. It was not considered as a food crop until the tenth to the twelfth centuries; prior to that, it was a medicinal plant (Chaudhari & Pandya, 2020). In its natural state, the taproot is woody. Breeding and selection, however, have made it possible for the grown form's root to thicken and become succulent. Around the seventeenth century, selections for orange roots were made in Holland and later in France; these selections led to the development of modern European cultivars (Yang *et al.*, 2018).

In the first year of carrot, growth is spent vegetatively, during which time it creates an expanded hypocotyl and a noticeable taproot. The root is made up of an external cortex predominately made of phloem and an internal pith or core made of xylem (Waisen *et al.* 2021). Higher quality of carrots is linked to roots that are primarily made of cortex and include less pith. Colour is also helpful; a comparable deep orange hue is related to good quality (Vicente *et al.*, 2022).

Carrots have many benefits a the promote healthy vision, make the sugar in the body to be balanced, manages weight, reduce cancer risks, regulate blood pressure,lowers

the diseases of the heart, increase immunity, and boosts the health of the brain (Suvendu, 2023). Its roots have a pleasant taste that is brought on by a high concentration of soluble carbohydrates. The increased carotene content in the roots that contributes to their orange colour.

Dietary fiber, vitamins, and carotenoids can all be found in carrots. They also include nutrients and antioxidants (Surbhi et al., 2018). Carrot is used as a raw ingredient in a number of commercial food items, including purees, preserves, juices, and infant food, in addition to being eaten directly, either raw or cooked (Tyler & Zhongli, 2019).

Teixeira *et al.* (2016) carrots are raw, cooked, steamed, fried, sautéed, pickled, roasted with meats, and fresh. According to Teixeira *et al.* (2016), they can be prepared for canning, freezing, and slicing for inclusion in soups. Additionally, the produce is prepared for juice. A carrot grown for eating has between 40 and 50 percent sugars and roughly, 11% dry matter. The high amount of β -carotene is what gives the orange colour. Carrots are recommended as a treatment for night-blindness, which is caused by a lack of vitamin A (Suvendu, 2023) because β -carotene is a precursor to vitamin A.

2.2 Constraints to carrot production

The problem of poor quality seed is widely spread, because of poor enforceability of norms of ensuring quality of the seed material (Gupta & Jain, 2003). Furthermore, cold storage facilities are also lacking for this crop, which leads the sale of produce at low prices during peak season. Lack of enough storage and processing facilities reduces their quality (Chaudhari *et al.*, 2020).

People growing carrots are facing problems in hiring casual labour for carrying out farm activities such as weeding, harvesting, among others (Tyler & Zhongli, 2019).

Lack of enough labour during peak load periods was a big problem and it affected the yield of carrots per acre negatively. In addition, the financial requirement also high and farmers' financial resources are not enough to satisfy labourers' demand for increased wages (Suvendu, 2023).

As reported by from Chaudhari and Pandya (2020), the major constraints faced by the carrot growers in taking on improved carrot production technology were; unavailability of improved seed 100%, do not get remunerative price of production 84.37%, shortage and high wages of labour 80%, high cost of fertilizer 76.87%, high production cost 68.75 %, inadequate finance 65%, high cost of seed 59.37 % and attack by nematodes 55.00% which were ranked from I to VIII respectively (Tyler & Zhongli, 2019). It can be inferred from Tyler & Zhongli that unavailability of improved seeds, do not get remunerative price of production and shortage and high wages of labour are the major problems.

Carrot production is highly affected by plant-parasitic nematodes (*Meloidogyne* spp) worldwide (Iqbal, *et al.*, 2018). The developing countries especially in the Asia and subsaharan Africa are under threat as 60% of the carrot plantations are heavily infestated with nematode spp especially the root knot nematodes (Abiodun, *et al.* 2024).

2.3 Root knot nematodes as a constraint to crop production

Gregory, (2017) revealed that plant-parasitic nematodes are the main obstacles to crop production across the globe. Symptoms of the root-knot nematodes include the yellowing and withering of carrot leaves, which affect the growth of crops, particularly vegetables. Other signs of the infections include carrot root lesions, galling of roots and tubers, and reduced development. These symptoms expose the plant to worse conditions leading to great loss of plant yield. Additionally,

Meloidogyne species were listed as one of the top ten parasitic nematodes that impacted crops (Mesa *et al.*, 2020). This was after an assessment of the threat of *Meloidogyne* spp. to crop production with a specific emphasis on Africa had been conducted. In a related development, Nurul (2016) reported that, among the many genera of nematodes, *Meloidogyne* spp. causes great loss of crop yields amounting to billions of dollars on annual basis. The study revealed that nematode damage was one of the major challenges faced by farmers worldwide.

Zhou *et al.* (2016) revealed that the root-knot nematode is one of the most aggressive and harmful nematode pests in both tropical and subtropical crop producing zones, based on the substantial financial damages incurred. They listed over 200 plant species as possible hosts, including herbaceous and woody plants of both monocotyledone and dicotyledone species, and classified the parasitic nature as an obligatory root parasite. It was discovered that the primary agronomically significant parasites responsible for at least 90% of all damage were *Meloidogyne incognita*, *M. javanica*, *M. aranaria*, and *M. hapla* (Yigezu *et al.*, 2021). Castagnone-Sereno said that 22 of the almost 100 *Meloidogyne* species now known to science are said to be found in Africa.

The only species that affects carrots cultivated on soil in the Nilgiris and Kodaikanal highlands of Tamil Nadu, according to Seenivasan (2017), is the root knot nematode. Root knot nematodes are sedentary root endo-parasitic nematodes, according to (Kumar & Yadav, 2020), whose aggressiveness and infection are seen in the second stage juvenile (J2). They explained how nematodes infect plants by entering vascular tissues through the developing tips of roots and generating a number of multinucleate large cells. This causes the tap roots to digitate, constrict, and produce galls, which causes plant stunting and mortality.

According to Khanal & Land, (2023) warm weather favours the nematode attack on their hosts. Nematodes also thrive well in moist soils with good aeration as well as sandy soils where the host plants are present. The study revealed that nematodes occur mainly in the upper layers of the soils, and have a tendency of following the roots in the deep layers of the soil. The study also reported that in unfavourable conditions the eggs can remain viable for a long time ranging from months to years. Other workers have also reported that the vegetables especially carrots display malformed, stubby roots (Bonniel, 2018).

Damages caused by *Meloidogyne* in carrots is observed by many galls near the lenticels due to over staying in the field (Gorny, 2019). They further reported that carrot damage is shown by physical tuber distortions and blemishes, which lower their market value.

The damage inflicted on the plants by root-knot nematodes is summarized in their life cycle by Saucet *et al.*, (2016) as follows: The life cycle of root knot nematodes starts with the eggs that are laid in the soil or plant tissues making the plant have hairy roots structures, ugly and distorted with tough galls and thick skin. The eggs hatch into larvae stage as juvenile 1 within the egg shell, juvenile 1 moults to juvenile 2 this moulting process occurs after 10 days, there after the juvenile 2 ruptures the egg shell and come out. They then find their food source by sensing substances and moult to juvenile 3 and juvenile 3 metamorphoses to 4 juvenile stages and then adults.

2.4 Management of root-knot nematodes using organic pesticides in crop production

Durán-Lara *et al.* (2020), reported that organic gardening is useful for growing healthy foods without the application of the nematicides and but instead with

biological control agents which are not harmful to humans and the environment. Roskopf *et al.* (2020), stated that the use of organic amendments, resistant varieties, soil solarisation and biological control agents show a potential to suppress the population of nematodes in different cropping systems. In a study involving the use of organic pesticides, Nwankwo *et al.* (2016) revealed that, the neem leaf extract of five different levels – (100%, 80%, 60%, 40%, and 20%) of concentrations performed better in controlling root-knot nematodes in cowpea. The study further reported organic pesticides to have increased the growth and yields of plants than those treated with carbofuran concentrations which were (1000, 750, 500, 250 and 100) mg/L at a rate of 5mls per treatment. Therefore, it is advisable to use low concentration of inorganic pesticides. It has been reported that low concentration of agrochemicals increases the respiratory activities in the soil hence improve growth due to good rhizosphere for mycorrhizal associations (Hage *et al.*, 2019).

Relatedly, Li & Chen (2017) reported that an increase in neem extract toxicity repels and kills nematodes thus less or no infection on the root tips to cause forking. Khanna *et al.* (2022) study revealed that a variety of soil amendments, including compost and green manures, organic and inorganic fertilizers, botanicals, and bio-agents like as soil bacteria and nematophagous fungi, can be used to control root knot nematodes. All have been shown to offer higher yields and more satisfactory results in terms of nematode population reduction.

According to Njenga *et al.* (2019), neem seed extract (*Azadirachta indica* L) was utilized to manage the sweet gourd root knot nematode *Meloidogyne javanica*. In contrast to the tree bark extracts, the same extract was found to be fatal to the juvenile *M. javanica*.

A study conducted by Ladi *et al.* (2019) revealed that, fresh or dry neem leaf extract can totally prevent the hatching of larva from the fresh egg masses of *M. incognita*. The study further noted that active ingredients from fresh neem extract were more readily available for action than those from the dry extract. However, given a span of 24 hours the ingredients from both the fresh and dry extracts would be equally available for action. The study further asserted that, fresh and dry neem leaf extract had a significant reduction on root knot infection indices on tomato. However, furthermore the study revealed that garlic extract demonstrated greater potential than neem leaf extract in the control of root-knot infection of tomato in vivo.

According to Gautam *et al.* (2019), when applied at varying concentrations of 20, 10, 5, 1, and 0.5%, the leaf extract of orange berries (*Glycosmis pentahylla*) and the bark of Kurchi (*Holarrhena antidysenterica*) demonstrated strong nematoidal properties. The extract also showed greater ovicidal activity on egg masses and larvicidal activity on juveniles of *M. incognita* (98.1%-35.9% inhibition) than the bark extract of *H. antidysenterica* (84.3%-28.3% inhibition) at the same concentration. Acheuk *et al.*, (2022) reported that in the USA, Canada and European countries, formulations of neem extracts have been applied in crude form at local and small farm level producing desired results.

According to Resha & Rani (2015), extracts obtained from fresh green leaves reduced egg hatching to a maximum and killed the juveniles to a significant level in Okra plants. The mortality rate in the green fresh leaves was followed by that of fresh green seeds and then of the dry seed extracts respectively. In pot settings, fresh seed powder outperformed fresh leaves and dry seed powder in terms of plant growth and decreased root knot formation. Local farmers can employ neem seed extracts to combat root knot nematodes and prevent the bioaccumulation of synthetic nematicides (Singh, 2017).

According to Aji *et al.*, (2024) test plant extracts of neem, chilli pepper, ginger and garlic on the micro plots suppressed the population of *M. javanica* and *M. incognita* in tomato where different levels of 250, 500, 750 and 1000ppm concentrations were used and 5mls from each extract was applied per tomato stand. The study revealed that at 1000ppm concentrations the extract applied prevented completely 100% root knot juveniles in tomato plants.

Sharma *et al.*, (2017) study revealed stated the cost-effective, and environmentally less-toxic measures for managing this pest which pose great threat in farming, they concluded that neem extracts can effectively manage root knot nematodes. Furthermore, a study conducted by Hay & Pethybridge (2005) asserted that, the nematode control strategies that make use of sustainable environmental and productive resource efficiency practices could aid in taking-on organic agriculture as a sustainable food production system.

Traditional agricultural methods and small-scale agriculture in the tropics and subtropics have been crucial in reducing the incidence and harm caused by root-knot nematodes in a sustainable manner, according to Mollier *et al.* (2017). To effectively manage root-knot nematodes in small scale farming, other contemporary cultural and low-input techniques can be investigated and implemented. Most studies reviewed here have showed effect of using neem extracts, ginger, garlic and chilli paper on management of nematodes in tomatoes and gourd plants. However, there no study that has revealed the effect of using neem extracts on management of nematodes in carrots.

2.5 Effects of organic pesticides on the tuber quality of carrots

According to Dlugosz *et al.*, (2013), carrots free from root-knot nematodes possess proper growth of tap root, smooth skin with no digitations and give high yields. The

study also noted that the use of foliar applied bio stimulators can improve overall environmental conditions necessary for the growth of plants. It has also positive influence on the quality of the raw materials as well as plant resistance to harsh environmental conditions. Growth stimulators influence positively the parameters of the carrot roots in terms of quantity and quality. Relatedly, Reddy (2015), assert that susceptible host plants exposed to neem formulations and ethanol registered remarkable root gall incidences. According to Sharma (2023), broccoli residues and *Trichoderma* inoculant were able to decrease root-knot nematode population as they lead to no galling hence increasing yields in carrots.

According to Bender (2021), the marketable yield of organic carrots is 11% higher than that of conventionally grown carrots. They also expressed that, the contents of B-carotene, vitamin C and nitrogen are significantly lower in organically grown than in conventionally grown carrots.

2.6. Management of root-knot nematodes using neem tree extracts

Neem oil is a vegetable oil that is made from the fruit and seeds of the neem tree. It is well-known for its unique ability to improve human health and repel nematodes. High amounts of several substances, including *nimbin*, *nimbidin*, *azadirachtin*, *salannin*, *thionemon*, and *meliantriol*, are found in neem seeds, leaves, and bark. These are used to control nematodes with success. According to Chaudhary *et al.*, (2017), neem properties have been well documented as nematicides and antifeedant. The knowledge concerning its use as hormones, fungicides and nematicides is well documented (Adusei & Azupio, 2022) .

These properties are expressed when various parts of the plant, namely, the leaves, seeds, cakes and fruit extracts are utilised. The most outstanding component of the

neem is *Azadirachtin* which greatly influence negatively the feeding behaviour and development of nematodes (Petrikovszki *et al.*, 2019).

According to *Adusei & Azupio*, (2022), products extracted from neem tree are very effective as a control measure as they greatly reduce phytonematodes, thereby improving the health conditions of plants. For instance, the *Azadirachtin* reduces the hatchability of nematode eggs, and also renders the juveniles immobile at the time when they are supposed to search for the hosts. Adverse effects caused by the synthetic pesticides on the environment more especially the toxicity on the non-target organisms has led to increased development of organic pesticides. *Azadirachtin* is considered a safe pesticide which could not cause environmental risks and would not cause ecological problem in the microbial community in soils as it degrades very fast in water or light in a period of about 100 hours (Henderson & Lankoski, 2019). Similarly, the fact that *Azadirachtin* affects the nematodes' chemo receptors which deter the nematodes from feeding on the host plant, as well as the damage inflicted on the nematodes' tissues, including the muscle, fat and gut cells it is regarded as a strong antifeedant (Mordue & Nisbet, 2005). At 2, 4, 6, 8, and 16 weeks after being applied to the soil, the impact of the neem plant's leaves and cake on the growth of root knot nematodes was examined.

All treatments showed a considerable decrease in the quantity of egg masses and gall incidence at 16 weeks. However, after the neem leaves and cake were stored for eight months in ambient conditions, their success in the control decreased over time. The neem leaves' ability to repel root knot nematodes remained constant, but the neem cake's decreased after eight months (Rizvi *et al.*, 2015).

According to *Errico et al.* (2023), *Azadirachtin* did not control nematodes completely when exposed to higher doses but the exposure of juvenile2 to higher doses resulted in the multiplication of immobile J2 for *Aza Nema* and *Azadirachtin*. They further

concluded that *Azadirachtin* 'A' concentration remained stable regardless of the formulation type tested. Neem based formulations that is, Neema and Neema plus were highly effective on the developmental inhibition of root knot nematodes (Zeshan *et al.*, 2019).

A study by Kankam & Sowley (2016), investigated the efficacy of neem products which included, 20g of power obtained from the leaves of the neem plant, 20g of the neem seed and 20g of the neem cake against root knot nematodes. The results revealed that 20g of neem leaf powder reduced nematodes population leading to reduced number of galls in Chilli pepper.

The cake derived from neem and *Glomus* fasciculation gave promising results for long term control of nematodes and at the same time acting as efficient source of nutrients for tomato growth hence reduced nematodes multiplication and gall incidence (Soumya & Kumanand, 2021).

However, according to Henderson & Lankoski (2019), *Azadirachtin* can easily get degraded when exposed to extremes of the environmental factors such as heat, moisture, air and acidity as well as enzymes found in foliar surfaces.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

A field experiment was conducted during two rainfall seasons of 2019B and 2020A at Kyambogo University farm. The University is located in Kampala Capital City, Central Uganda, and 6 km along Kampala - Jinja Highway from the City centre. Kyambogo University is situated at 1194m above sea level on latitude 0.25° south and 20.59 north and longitude 32.63° east. The site receives a bimodal rainfall of averagely 1100-1650mm that lasts for 8month from march through june and short rains from October to December and minimum temperatures range of 20-25° and maximum range of 25-27° (Nielsr, 2021).

3.2 Preparation of the planting media

Loam soil, well decomposed manure and sand soil were collected and mixed in a ratio of 2:2:1 respectively. The composite media were sterilized by steaming at 65°C for 30minutes then left to cool for 72hrs. After cooling in a screen house, the soil was filled in 25 buckets in the quantity of 5kg of soil per bucket, thus ready for planting and placed in an open field.

3.3 Variety of carrot and field practices

Seed of Nantes carrot variety was obtained from East Africa Seed Company in Container Village, one of the Seed suppliers in Kampala. The variety was chosen because of its early germinability and great adaptability to rainy season, short production cycles and can be grown in all altitudes and gives good yield of 20 to 36 tonnes/acre

(https://market.ug/products/seeds-seedlings-and_suckers/seeds/carrot-nantes-50gm-high-yielding-carrots-with-straight-long-cylindrical-roots Retrieved from google 10/06/2025). During planting, 10 seeds were sown in each bucket and three weeks after germination they were thinned to one plant per bucket, weeded whenever weeds would appear and watered when the moisture in the planting media was low after physical observation. Two litters of water were added whenever it was insufficient in the buckets.

3.4 Source, preparation of inoculum (*Meloidogyne* spp) and inoculation of the experiment with Root-knot nematodes

The inoculum of root-knot nematodes (*Meloidogyne* spp) was obtained from the mature carrot tubers which were grown in the soils infested with root knot nematodes at Kyambogo University farm. The carrot tubers were harvested after 90 days and cut into small pieces of 1mm³. A weight of the carrot cut pieces of 100g each (about 1000 juveniles), was mixed with 500mls of water and applied in each bucket containing the growing carrot plant at a depth of 2.5cm and a distance of 2cm away from the plant. Therefore, about 1000 juveniles were inoculated in each set of buckets (Nazim *et al.*, 2003) around the growing carrot plant while the other set of plants was to serve as the check so it was not inoculated (Negative Control).



Plate 1: Carrot tubers with symptoms of *Meloidogyne* spp infestation



Plate 2: Inoculating carrot tubers with *Meloidogyne* spp

3.5 Source and Preparation of neem leaf extract

Fresh neem leaves were obtained from the neem tree of 15years of age located at the Faculty of Agriculture, Kyambogo University. *Azadirachtin* leaf extract of different concentrations of 15, 25 and 35g was prepared. Each weight of azadirachtin leaves (15, 25 and 35)g was crushed using a mortar and a pestle and mixed in 100mls of distilled water. The mixture was left to settle for 4 hours before use.



Plate 3: Neem leaves being weighed to varying weights of 15, 25 and 35g



Plate 4: Preparation of neem leaf extract

3.6 Experimental design and treatments

A Completely Randomised Block Design (CRBD) was set and treatments replicated five times. Treatments composed of three levels of concentration of *Azadirachta indica* leaf extract (i.e 15, 25 and 35g) and a positive control (distilled water) and a negative control (no inoculum). Equal weight (5kgs) of the growing media (soil) was loaded in 25 buckets each and water applied in the bucket containing the soil. This was followed by sowing 10 seeds of carrots in each bucket. The buckets were spaced at a distance of 90cm x 90cm. After 21 days from planting, carrots in each bucket were thinned to one plant. on the 28th days after planting, all buckets with plants were randomly labelled with the assigned treatments. After 14 days from inoculation and 42 days from planting, 5mls of each treatment was applied. These included different concentrations of neem leaf extract and distilled water applied at an interval of 14 days up to the 70th day of inoculation.

3.7 Determining the effect of *Azadirachta indica* leaf extract of different concentrations in controlling root-knot nematodes in carrots

Data collection on number of leaves per plant and height of the shoot was carried out at an interval of 14 days from the 56th day to the 84th day after treatment application (8 weeks after planting to 12 weeks). The data was collected 3 times up to the period when plants had reached their physiological maturity. However, data on weight of fresh leaves, weight of carrot tubers, length of carrot tubers and girth of carrot tubers was collected once on the 84th day after harvesting when the plants had reached their physiological maturity. The dry weight of leaves was recorded 48 hours after drying the fresh leaves in the oven at a temperature of 60°C.

The following parameters were used as indicators of the efficacy of neem leaf extracts on root knot nematodes in carrots:

3.7.1 Number of standing leaves on the plant

The number of standing leaves was determined by counting and recording the number of live leaves including those that were 50% open. This was done at an interval of 14 days after the treatment application (8 weeks after planting). (Megueni *et al.*, 2017).

3.7.2 Height of the shoot

Five plants were randomly sampled and height of each plant was measured from the collar region to the apex of the tallest leaf using a meter ruler at an interval of 14 days after the application of treatments (Megueni *et al.*, 2017).

3.7.3 Fresh weight of leaves

Determining the weight of fresh leaves was done on the 84 days after inoculation when plants had reached their physiological maturity, by removing and tagging carrot plants as per the treatment from the growing media. The shoot was broken off from the stem, placed in aluminium foil paper and carried to Crop Science laboratory at the Faculty of Agriculture, Kyambogo University. Loose soils that were attached on the shoot system were washed off gently under running tap water, and the shoot wiped dry with soft towel, and then weighed using a digital electronic scale (Joon & Jung, 2019).

3.7.4 Dry weight of leaves

To determine dry weight of carrot leaves, plant samples that had achieved their physiological maturity were removed and tagged as per treatment from the growing media. The shoot was broken off from the collar region of stem, placed in aluminium foil paper and carried to Crop Science Laboratory at the faculty of Agriculture, Kyambogo University. Loose soils that were attached on the shoot system. The samples were once again wrapped in aluminium foil paper, labelled and heated to

temperatures of 80°C in the oven for 48hrs and weighed. The weight was determined using a digital electronic scale (Joon & Jung, 2019).

3.7.5 Fresh weight of Carrot tubers

The samples of fresh carrot tubers were removed from the growing media for each treatment, washed and the root hairs cut off, and the tubers wrapped in aluminium foil paper and carried to the laboratory. Thereafter the fresh weight of the carrot tuber was determined using a digital electronic scale (Megueni *et al.*, 2017).

3.7.6 Length of tubers

Length of five tubers per treatment was measured using a thread and meter ruler Megueni *et al.* (2017) and Joon & Jung (2019).

3.7.7 Girth of the carrot tuber

The girth of carrot tubers was determined by measuring the circumference of the tuber using a thread that was first placed around the carrot tuber, 2cm from the stem. The thread was then laid onto the meter ruler to read off the girth in centimetre (Quezada, 2010).

3.8 Determining the effect of *Azadirachta indica* leaf extract on carrot tuber galls and forks incidence

The gall and forking incidence on carrot tubers were assessed on 84 days after inoculation when the plants had achieved physiological maturity.

3.8.1 Gall incidence

Determination of the gall incidence involved counting and recording the tumorous type of expansions emerging from the tuber and those tumours (knots) on root extensions approximate 4cm away (Noling, 2019).

3.8.2 Forking incidence

The forking incidence of carrots was assessed by counting and recording the number of branches or outgrowth that had emerged from the lower end of the tuber (Kristina, 2020).

3.9 Statistical data analysis

The data were entered in excel then imported into statistical package Genstat 15th version. The data was statistically tested for normality (Ghasemi& Zahediasl, 2012) and homogeneity by Balett test (Balett, 1937). First and second season data were pooled so that the underlying data generating processes are similar enough across both seasons to allow for a combined analysis. Data was analysed by analysis of variance for both objectives. This assumed that the key variables being measured, the way they are measured, and the relationships between them are consistent across both time periods. Mean separation was at LSD 5%. However, Results for objectives two were established graphically in Microsoft excel 2013 with standard error represented by bars over the mean.

CHAPTER FOUR: PRESENTATION OF RESULTS

4.1. The effect of *Azadirachta indica* leaf extract concentration on growth and yield of carrots

4.1.1. Effect on carrot leaves

The results of the study revealed that *Azadirachta indica* leaf extract concentration did not affect number of standing leaves in carrots was significant ($P = 0.033$) (Table 1). The highest number of standing leaves was obtained in plots treated with neem leaves (15g) treatment while the lowest was from plots treated with distilled water (Table 1).

The results of the study revealed that *Azadirachta indica* leaf extract concentration did not affect shoot length significantly ($P = 0.204$) (Table 1). The highest shoot length was seen in plots treated with neem leaves (35g) treatment while the lowest was from plots treated with distilled water (Table 1).

The results of the study revealed that *Azadirachta indica* leaf extract concentration affected fresh leaf weight significantly ($P = 0.001$) (Table 1). The highest fresh leaf weight was recorded from plots treated with neem leaves (35g) treatment while the lowest was from plots treated with distilled water (Table 1).

The results of the study revealed that *Azadirachta indica* leaf extract concentration affected dry leaf weight significantly ($P = 0.001$) (Table 1). The dry leaf weight was obtained in plots treated with neem leaves (35g) treatment while the lowest was from plots treated with distilled water (Table 1).

Table 1: The effect of *Azadirachta indica* leaf extract of different concentrations in controlling root-knot nematodes on carrot leaves

Treatment	No of Standing leaves on on carrot plant	Shoot Length (cm)	Fresh Leaf Weight (g)	Dry Leaf Weight (g)
Negative Control	8.60 ± 0.98 ^{ab}	37.14 ± 2.295 ^a	22.41 ± 3.520 ^{ab}	3.5 ± 0.743 ^a
Neem leaves (15g)	12.30 ± 1.832 ^c	34.72 ± 1.031 ^a	29.19 ± 3.064 ^{bc}	5.52 ± 0.575 ^b
Neem leaves (25g)	11.60 ± 1.222 ^{bc}	34.1 ± 2.420 ^a	37.14 ± 4.655 ^c	5.83 ± 0.590 ^b
Neem leaves (35g)	9.80 ± 0.827 ^{abc}	37.4 ± 1.720 ^a	37.61 ± 3.924 ^c	6.62 ± 0.578 ^b
Distilled water	7.80 ± 0.249 ^a	32.35 ± 0.85 ^a	16.54 ± 1.306 ^a	2.83 ± 0.194 ^a
P. Value	0.033	0.204	<.001	<.001
d.f	4	4	4	4

Means followed by the same letter in a column are not significantly different (P = 0.05) as determined by fisher protected l.s.d.

4.1.2. Effect on carrot tubers

The results in table 2 indicates that, there was a noticeable difference between the highest level of neem leaf extract concentration (35g) and positive control ($P<0.001$). Similarly, there was a marked contrast between negative and positive control. However, there was no clear boundary between the lower levels of neem leaf extract concentrations (15g and 25g) and the positive control ($P<0.001$).

The results from the tuber girth showed a considerable discrepancy between the highest level of neem leaf extract concentration (35g) and lower levels of neem extract concentrations (15g and 25g) as well as the positive control ($P<0.001$). Relatedly, there was a striking contrast between negative and positive control.

The tuber length of carrot results reveal that the higher levels of neem leaf extract concentrations (25g and 35g) showed significant difference with the positive control and the lowest level of neem leaf extract concentration (15g). There was also clear differentiation between the lowest level of neem leaf extract concentration (15g) and the positive control. It is also worth-noting that there was a complete change between the negative and positive control all at ($P<0.001$).

Table 2: Effect of neem leaf extract of different concentrations on carrot tuber weight, girth and length

Treatment	Tuber Weight (gm)	Tuber Girth (cm)	Tuber Length(cm)
Negative Control	96.12 $\pm$ 16.89 ^b	12.07 $\pm$ 0.918 ^{bc}	17.57 $\pm$ 0.669 ^c
Neem leaves (15g)	59.05 $\pm$ 8.027 ^{ab}	10.11 $\pm$ 0.880 ^{ab}	13.16 $\pm$ 1.189 ^b
Neem leaves (25g)	68.21 $\pm$ 12.44 ^{ab}	11.05 $\pm$ 0.581 ^b	16.22 $\pm$ 0.808 ^c
Neem leaves (35g)	93.94 $\pm$ 7.185 ^b	14.38 $\pm$ 0.658 ^c	18.08 $\pm$ 0.516 ^c
Distilled water	33.98 $\pm$ 5.767 ^a	7.8 $\pm$ 0.918 ^a	8.65 $\pm$ 0.927 ^a
P. Value	<.001	<.001	<.001
d.f	4	4	4

Means followed by the same letter in a column are not significantly different ($p = 0.05$) as determined by fisher protected l.s.d.

4.2. Effect of *Azadirachta indica* leaf extract concentration on suppression of galling and forking incidence on carrot tubers

4.2.1. Effect of neem leaf extract on the galling incidence on carrot tubers

Results in figure 1 indicated that, there was a complete change between the higher neem leaf extract concentrations (25g and 35g) and the lowest neem leaf extract concentration (15g) as well as positive control. There was also a substantial shift between the negative control and the lowest neem leaf extract concentration as well as the positive control ($P < 0.001$).

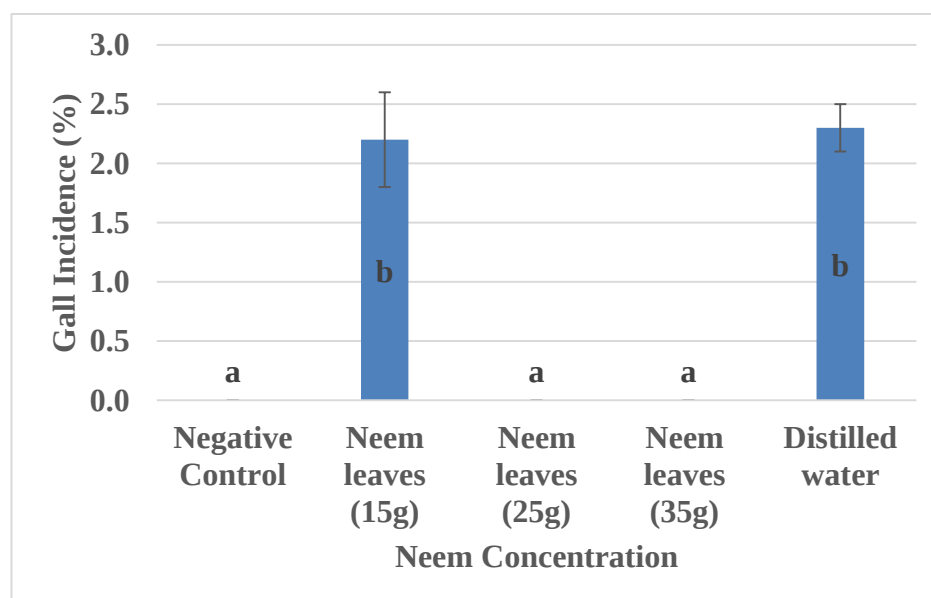


Figure 1: Gall incidence on carrot tubers for different neem leaf concentration

4.2.2. Effect of neem leaf extract on forking incidence on carrot tubers

Results of the study in figure 2 revealed excessive branching (forking incidence) in positive control and the lowest level of (15g) of neem leaf extract concentration. There was significant difference between the higher levels of neem leaf extract concentrations (25g and 35g) and the positive control. The significant difference was

also observed between the lowest level of neem leaf extract concentration and the positive control. Similarly, there was significant difference between higher levels (25g and 35g) and the lowest level (15g) ($P < 0.001$).

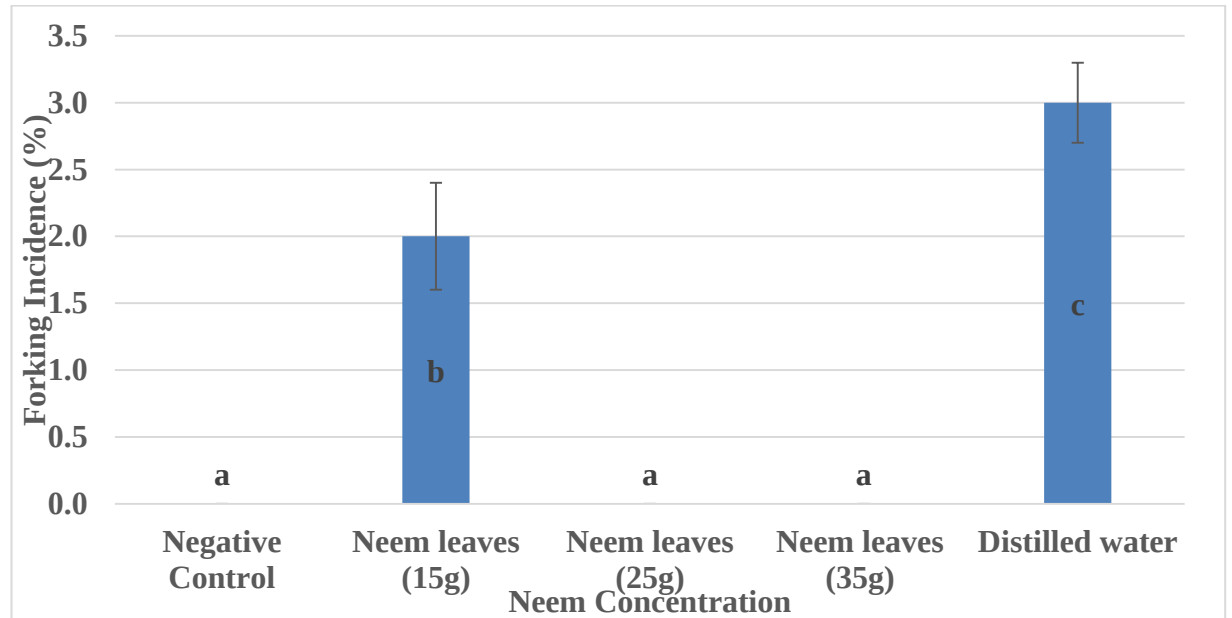


Figure 2: Forking incidence on carrot tubers treated with different neem leaf extract concentration

CHAPTER FIVE: DISCUSSION OF RESULTS

5.1 The effect of *Azadirachta indica* leaf extract concentration on growth and yield of carrots

Findings on the effect of *Azadirachta indica* leaf extract at various concentrations in preventing root-knot nematodes on carrot leaves revealed that there was insignificant difference between the number of standing leaves in carrots under the negative control (no inoculum applied), positive control (inoculum applied), and highest concentration level of neem leaf extract (35g). Relatedly, there was no significant difference in all the concentrations of neem leaf extract (15g, 25g, and 35g). From the results it can be found that the presence or absence of nematodes has little or no effect on the number of standing leaves in carrots. That could be the reason for non-significance registered above. In the same line, Andresen & Gedergreen (2010) observed that when root-knot nematodes attack the roots; the plant might be in position to repair the damage or withstand the attack before the leaves are affected (Vandegehuchte *et al.*, 2010). Alternatively, the leaves may be affected at certain level of incidence and severity which in this case were not established by the study (Teixeira *et al.*, 2016).

The height of the shoot did not register any significant difference across all the treatments. However, the longest shoot of carrot was recorded where 35g of neem leaf extract was applied. This might have been due to high concentration of saponins and polyphenol compounds in neem leaf extracts that repel and effectively kill pests that attack plants. The results of this study agree with Gayatri & Rajani (2014) who reported a significant improvement of Brinjal medicinal plant shoot system.

For fresh weight of leaves, non-significance was observed in the negative and positive control. Therefore, the plausible explanation is that the attack on the roots

had little effect on the leaves of carrot plants (Anita & Selvaraj, 2011). This trend of results is similar to that of the standing leaves discussed above. Also, non-significance occurred among the neem leaf extract treatments. However, there was an observable difference between the neem leaf extract treatments and both the negative and positive control. The significant difference could have been brought about by the neem leaf compounds since they are reported to increase the biomass content in the leaves. Henderson and Lankoski (2019) reported that *Azadirachtin*, *nimbin*, *nimbidin*, *nimbinene* and *azadrone* that are antifungal, antibacterial, antiparasitic rendered the soil media free of pathogens. This therefore increased the biomass of the plants because there were no interruptions by pathogens on assimilation of nutrients by plants, hence more biomass. This study concurs with finding of Gayatri & Rajani (2014) who reported an increase in growth parameters when a high concentration of neem and tea extracts were applied on Brinjal medicinal plant.

The results for the dry weight of carrot leaves follow a similar pattern to those for the fresh weight of the leaves. The negative and positive controls did not significantly differ from one another, in other words. The neem leaf extract treatments significantly differed from the positive and negative controls, as well.

The occurrence, could be as result of the findings reported by Gayatri & Rajani (2014) that *Azadirachtin*, *nimbin*, *nimbidin*, *nimbinene* and *azadrone* that are antifungal, antibacterial, antiparasitic rendered the soil media free of pathogens which eventually enable the plants to grow and produce biomass without interruption. The findings by Henderson & Lankoski (2019) and Kolapo & Kolapo (2021) have similar views that the increase in biomass was due to suppression of pathogens by neem leaf extract compounds besides the release and assimilation of organic minerals to facilitate partition of photosynthates to biological parts of the plant.

On the efficacy of *Azadirachta indica* leaf extract of different concentrations in controlling root-knot nematodes on carrot tubers, finding of the study indicated a significant difference between the highest level of neem leaf extract concentration (35g) and the positive control, also a significant difference was observed between the negative control and the positive control. This could have been due to choosing nematode free growing sites and nematode free planting materials. This finding agrees with Olsen (2011) who revealed that cultural farming practices greatly enhance reduction in the incidence and damage caused by root-knot nematodes. Fess & Benedito (2018) however, observed that neem leaf extract with a high concentration can be due to sufficient toxicological neem properties that suppress nematodes hence no disturbance, and influence optimisation on all nutrient that could nich the growth media and therefore more storage of carbon in the economic carrot part. The findings of this study are in agreement with Bawa *et al* (2014) who reported that 100% neem extract concentration repelled all nematodes in tomato plants hence high yield was achieved.

A very important difference ($P < 0.001$) was shown across all treatments on carrot stem girth, however, application of 35grams of neem leaf extracts resulted into wider stem girth compared to other treatments. In the same line, Ibukunoluwa (2012) revealed that this is attributed to presences of high mineralogical compounds (nitrogen, phosphorus, magnesium and potassium) along side suppression for pathogens. Therefore these attributes contributed to vigorous growth and enlargement of the economic plant parts to store assimilates in form of photosynthates. This study agrees with findings of Nwankwo *et al.* (2016) who reported that high neem leaf extract concentration significantly improved cowpea stem girth and general yield.

The tuber length of carrots indicated a significant difference between the higher levels of neem leaf extract concentrations (25g and 35g) as well as the positive control. Similarly a significant difference was observed between the negative control and the positive control ($P < 0.001$). In the same vain, Ibukunoluwa (2012) noted that this is attributed to high concentration of phosphorus since its directly involved in root cell differentiation and growth besides nematicidal properties of neem leaf extracts. The findings are in agreement with Bawa *et al.* (2014) who stated that 100% neem extract concentration repelled all nematodes in tomato plants hence high yield was achieved.

5.2. Effect of *Azadirachta indica* leaf extract concentration on suppression of galling and forking incidence on carrot tubers

Higher concentrations and the positive control significantly ($P < 0.001$) differed in the investigation to determine the impact of neem leaf extract on gall incidence. However, applications of the higher levels of neem leaf extract concentrations (25g and 35g) and the negative control resulted in non-appearance of galls on carrot tubers. This might have been due to high toxicity of the neem leaf extract that repelled and killed most of the nematodes and a nematode free growing media hence no attack on the carrots and no galls registered. Conversely plants that received the lowest level of neem leaf extract concentration (15g) and the positive control recorded high gall incidences. This might have been due to low toxic metabolites in 15grams that could not sufficiently repel and kill the adult females. Hence increased number of egg masses that were produced and hatched into nematodes. These nematodes started to feed onto carrot tubers resulting into a cross-talk between hormones, transcription factor to form galls. This study conquers with Uddin *et al.* (2003) who reported that an increase in the extract of neem toxicity repels and kills nematodes thus reduced or caused no galls.

The results of the study showed a significant difference ($P < 0.001$) between treatments on forking incidence. However, applications of the higher levels of neem leaf extract concentrations (25g and 35g) and the negative control resulted in non-production of forks on carrot tubers. This could probably be due to high toxicity of neem leaf extract and growing in a nematode free media hence no root tip destruction to cause forking. This finding agrees with Uddin *et al.*, (2003) and Westerdahl & Becker (2017) who reported that an increase in neem extract toxicity repels and kills nematodes thus reduced or caused no forking. Conversely plants that received the lowest level of neem leaf extract concentration (15g) and the positive control recorded high forking incidences. This might have been due to low toxic metabolites in 15grams that could not sufficiently repel and kill the adult females. Hence increased number of egg masses that were produced and hatched into nematodes. These nematodes started to feed onto carrot tubers resulting into a cross-talk between hormones hence developing multiple roots. This work supports Uddin *et al.*, (2003) who revealed that nematodes are repelled and killed by neem extract toxicity, which reduces or eliminates forks.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The general objective of the study was to assess the effectiveness of neem tree leaves (*Azadirachta indica*) extracts in the management of root knot nematodes in carrots. Specific objectives were to determine the efficacy of *Azadirachta indica* leaf extract of different concentrations in controlling root-knot nematodes in carrots, and assess its effect on carrot tuber quality.

The findings show that, application of the extract from neem leaves significantly improved ($P<0.05$) a number of standing leaves of carrots, weight of fresh leaves, weight of dry leaves, weight of carrot tubers, length of carrot tubers. However, neem leaf extract had little positive influence on the height of the shoot. The application of neem leaf extract at 35gram destroyed and/or repelled nematodes hence greatly boosting the number of standing leaves of carrots, weight of fresh leaves, weight of dry leaves, weight of carrot tubers, length of carrot tubers and shoot length. These effects were also observed in descending order at levels of 25grams, 15grams and in the positive control respectively.

The results of this study showed that application of *Azadirachta indica* (neem leaf extract) significantly improved ($P<0.05$) gall and forking incidence of carrot tubers. However, application of 35grams, negative control, and 25grams significantly suppressed nematode infestation on carrots than 15grams and positive control.

6.2 Conclusion

The following is concluded from the results of the study:

- The results of the study revealed that *Azadirachta indica* (neem) leaf extract significantly influenced several growth and yield parameters of carrots. Overall, 35g of neem leaf extract was most effective in improving both vegetative growth and tuber development in carrots.
- The findings of this study revealed that higher concentrations of *Azadirachta indica* (neem) leaf extract, particularly at 25g and 35g, were significantly effective in suppressing both galling and forking incidences on carrot tubers. These results suggest that higher doses of neem leaf extract possess potent nematocidal and growth-regulating properties, making them a promising botanical alternative for managing root-knot nematodes and related deformities in carrot production.

6.3 Recommendations

Based on results of this study, I recommend the following:

- Farmers in peri-urban and urban setting who are practicing bucket farming of carrots and other vegetables to adopt application of 35 grams of neem leaf extract paste per plant when controlling nematodes to increase yield or productivity.
- Further research is needed to compare 35 and 25 grams of neem leaf extract paste with common nematicides on market, to find out their efficacy in eradicating root-knot nematodes in carrots planted in vivo (controlled environment) or invitro.
- Research is still needed to compare neem cake and neem oil extracts to find out how they can effectively control root-knot nematodes in carrots.

- Further research is needed to compare neem leaf extracts in the management of root knot nematodes in carrots grown in buckets with those grown in the field.

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