EFFECTS OF DIFFERENT BIO-FUMIGANTS ON THE CONTROL OF ROOT-KNOT NEMATODES *(Meloidoygne javanica)*.

By

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DECLARATION OF ORIGINALITY OF RESEARCH

I hereby declare that this dissertation has been the result of my own efforts and investigations, and such work has not been presented elsewhere for any degree. All additional sources of information have been acknowledged by means of references.

Student's signature ……. Date …….

Supervisor's signature …… Date ……

ABSTRACT

Root-knot nematodes are one of the major economically important pests causing yield losses of up to 80% in many regions of the world including Zimbabwe. Control of nematodes has been mainly based on use of synthetic nematicides and these have negative impacts on the environment, as a result there is growing interest in alternative methods of management that are economically viable and non-polluting such as Bio-fumigation. An *In vitro* experiment was carried out in the 2018/19 growing season at Horticulture research Centre to investigate the use of different bio-fumigants (*Brassica juncea*, *Brassica carinata*, purple stem *Cleome gynandra* and green stem *Cleome gynandra*) at varying rates of 3, 5 and 10grams on the control of root-knot nematodes. The experiment was laid out as a 4×3 plus 1 factorial arranged in a Compete Randomised Design (CRD) with 13 treatments replicated 4 times. Nemacure®400Ec at 0.5ml/10mls water was used as a positive control. 100 juveniles of *Meloidogyne javanica* pure culture was obtained from Tobacco Research Board and were exposed to the different treatments and data was collected on the number of dead juveniles and number of eggs. Results show that there was significant interaction ($p \leq 0.01$) on the effects of bio-fumigants and rates on nematode mortality *Brassica juncea* at 10grams recording the highest juvenile mortality when compared to other bio-fumigants. There was no interaction ($p \leq 0.09$) between bio-fumigant type and rates on number of nematodes eggs. However, there was significant ($p \leq 0.01$) difference on the effect of the individual factors on number of nematode eggs recorded. *Brassica juncea* recorded the least number of nematode eggs followed by *Brassica carinata*, purple *Cleome gynandra* and green *Cleome gynandra* respectively. There was significant ($p \leq 0.03$) difference on the efficacy of different bio-fumigants rates on number of nematode eggs with 10grams recording the least number of eggs. Volatile compounds (ITCs) Isothiocynates produced from the hydrolysis of glucosinolates in plant tissue could have contributed to juvenile mortality and reduction in number of eggs recorded. It can therefore be concluded that use of *Brassica juncea* at 10grams as a bio-fumigant could be an alternative way of controlling root-knot nematodes.

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ACRONYMS AND ABBREVIATION

ANOVA -Analysis of variance

ITCs- isothiosynates

GLS- Glucosinolates

HRC –Horticulture Research Centre

LSD – Least Significant Difference

MYR-Myrosinase

RKN-Root-knot Nematode

Spp-Species

TRB- Tobacco Research Board

CHAPTER 1

INTRODUCTION

Root-knot nematodes are obligate endo-parasites and most destructive plant pests which limit agricultural productivity (Ibrahim, 2011). Nematode infection causes vascular damage which disturbs water and nutrient uptake (Sikora, 1990; Luc *et al*., 2005) and its feeding affects yield through the diversion of plant photosynthates to the nematode (Lazzeri *et al*., 2004). The reduction in root volume and function caused by the galling is also an important factor as the plant would not be able to forage effectively for nutrients and water (Hay *et al*., 2014). Plants affected by root-knot nematodes generally have areas of stunted, unthrifty plants that are often patchily distributed within a field. Heavy nematode infestation will cause premature plant wilting and plants tend to recover slowly when the crop is watered (Hay *et al*., 2014). Chlorosis (yellowing) and other symptoms of nutrient deficiency may also be apparent. Post-harvest losses occur in root and tuber crops because consumers are unwilling to purchase products that have been disfigured by nematodes (Luc *et al.,* 2005).*Meloidogyne* spp have being noted to form dynamic disease complexes with fungi and bacteria resulting in devastating disease incidences in cultivated plants (Bernard *et al* .,2017).

There are three most common and widely distributed species which are *Meloidogyne incognita, Meloidogyne arenaria and Meloidogyne javanica,* attacking more than 2000 species causing heavy losses in vegetable yields reaching up to eighty percent (Nchore *et al.*, 2011; Cetintas & Yarba, 2010).Currently, these harmful nematodes have been controlled using applications of broad-spectrum, synthetic soil fumigants (i.e.methyl bromide, metam sodium, and 1, 3-dichloropropene). These synthetic soil fumigants are highly harmful to humans as well as many

beneficial soil organisms (Schreiner *et al*., 2001; Cox, 2006). In addition many of these conventional soil fumigants are persistent in the environment, expensive, lead to pathogen resistance and cause ground contamination of surface and drinking water.

During the last decades, nematologists worldwide have been searching for better alternatives methods such as bio-fumigants in controlling root-knot nematodes (Salem, 2012; Salem, 2014, Salem *et al*., 2015). Bio-fumigation is the use of volatile chemicals released from decomposing plant material to suppress soil pathogens, insects and germinating weed seeds (Karavina and Mandumbu, 2012). Bio-fumigants are readily available, cheaper, safer because they are non-persistence in the environment (eco-friendly) and do not lead to pathogen resistance.

Plants in Caricaceae, Capparaceae, Moringaceae, Salvadoraceae, Tropaeolaceae and Brassiceae families also have been noted to contain glucosinolates (van Dam *et al*., 2009), these species contain alkaloids which are biocidal. In this study plants form the Capparaceae (cat´s whiskers) and Brassicaceae (mustards) were used these are high in glucosinolates which are sulphur and nitrogen compounds that occur naturally within these species (Karavina and Mandumbu, 2012). Glucosinolates are basically the precursors of (volatile chemicals) isothiocyanates (ITCs), organic cyanides, ionic thiocynates and oxazolidinethiones which have broad biocidal activity (Vig *et al.,* 2009) on bacteria, soil borne fungi and nematodes.

According to Brown and Morra, (2005) glucosinolates types in plant species are highly variable. Bio-fumigants used in this study include *Brassica juncea* and *Brassica carinata* which are dominated by 2propenly ITCs (Salem *et al*., 2012) these interfere with nematode reproductive cycles and have high cytotoxic activity on nematodes hence they are used as trap crops to control root-knot nematodes. *Cleome* species were noted to produce methyl, cleomin and glucocapparin glucosinolate these give rise to methyl ITCs (Silue *et al*, 2009) with various efficacious pest

control. According to Morra and Kirkegaard, (2002) methyl ITC cause inhibitory effects through interaction in proteins. Therefore it is against this background that this study was carried out to establish the effect of different bio-fumigant materials and rates on the control of root-knot nematodes.

1.1 Main objective

To evaluate the efficacy of bio-fumigants namely *Brassica juncea, Brassica carinata,* purple and green *Cleome gynandra* when applied at different levels for the control of root-knot nematode (*Meloidogyne* javanica).

1.2 Specific objectives

- To evaluate the effect of different bio-fumigants and rates on root-knot nematode mortality.
- To evaluate the effects of different bio-fumigants and rates on number of root-knot nematode eggs.

1.3 Hypotheses

- Different bio-fumigants and rates increased mortality of root-knot (J2) nematodes.
- Different bio-fumigants and rates reduced number of root-knot nematode eggs.

CHAPTER 2

LITERATURE REVIEW

2.1 Distribution and ecology of root-knot nematodes

The root-knot nematode (*Meloidogyne* spp.) comprises over 100 species, with *Meloidogyne javanica, Meloidogyne arenaria, Meloidogyne hapla,* and *Meloidogyne incognita,* which are a huge threat to agricultural crop production (Bernard *et al*., 2017). RKN falls under the Phylum; Nematoda, Order: Tylenchida, Family: Meloidoynidaea: Genus; *Meloidogyne and* Species*; Meloidogyne javanica* classification by *(*Karseen and Moens, 2006). Root-knot nematodes are known as warm climate species of *Meloidogyne* mainly found in temperate, tropical and subtropical regions which are warm, they are globally distributed species (Bernard *et al*., 2017).

Nematodes are generally free-living in marine, freshwater and soil environments but a large number of species are parasitic on different kinds of plants and animals (O'Halloran and Burnell, 2003). Nematodes favour temperature of 25-35ºC, temperatures below 10ºC are fatal for *M. javanica.* For optimum growth relative humidity ranging from 40 – 60% is ideal and less than that would result in lower nematode activity. Excessive application of fertilizers in soils can also affect nematode activity as it increase the electric charge of the soil (Khan *et al*., 2012), nematodes require pH of 5.8 – 6.5. Some parasitic nematodes are migratory and move in and out of root tissues, while some are sedentary and effectively don't move at all (Flint and Dreistadt, 1998).

In almost every soil sample, nematodes from five trophic levels namely bacteriovores such as rhabditids and cephalobids nematodes, fungivores that feed on fungi such as *Lotonchium* spp, plant parasites these feed on plant tissue include *Meloidogyne* species, predators that feed on

other nematodes which include nematodes in the order Mononchida and Dorylaimida and lastly omnivores that feed on algae and other soil living organisms such as *Dorydorella* spp are present (Yeates *et al*., 1993). Due to their biological diversity and particularly feeding habits, nematodes are an integral part of the food webs in soil ecosystems.

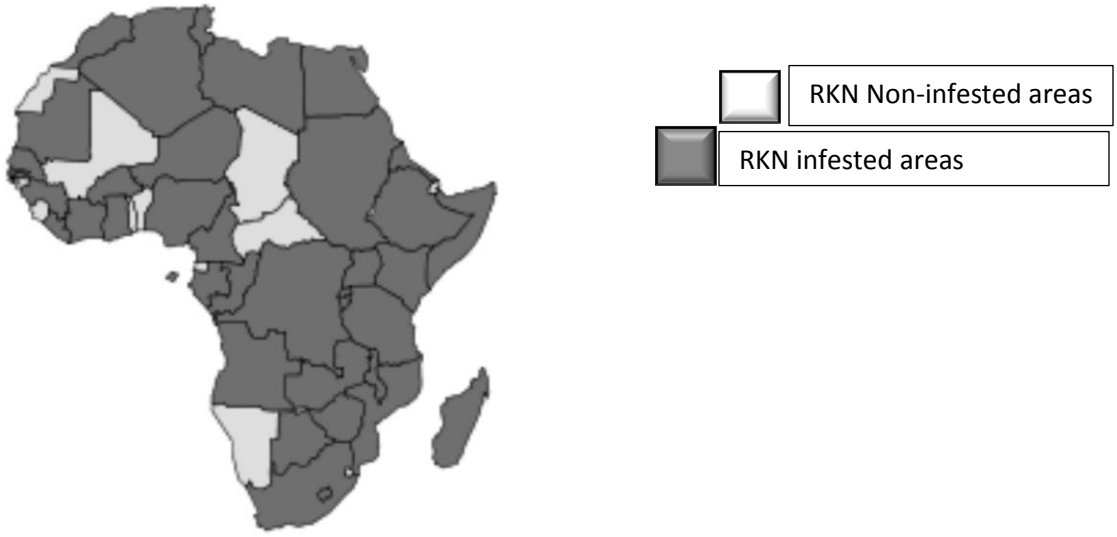

Figure 1: Distribution of plant parasitic nematodes (RKN) in Africa.

Source: http://www.infonet-biovision.org

2.2 Lifecycle and morphology

The lifecycle of *Meloidogyne* spp. involves four developmental stages including larval stage 1 (within the egg), larval stage 2 (migratory/ inside plant), larval stage juvenile 3 (sedentary), larval stage 4 (sedentary) and last stage the adult stage (sedentary), (Bernard *et al*., 2017).

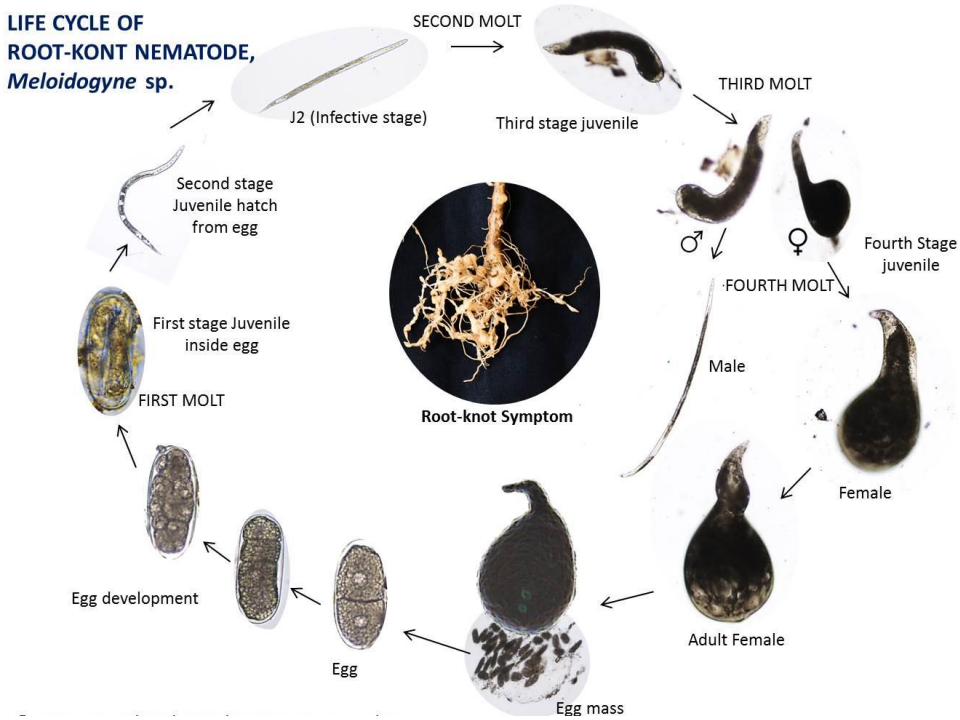

Figure 2: Root-knot nematode lifecycle (Adapted from Haque, 2012).

2.2.1 Egg

Life cycle begins with the egg which has an elongated oval shape with rounded ends (ellipsoid shape) (Galleti, 2008) with a length (5.71µm) and width (2.83µm). Eggs are found in the gelatinous matrix called the egg mass which prevent moisture loss. Eggs are usually deposited on plant roots or within the galls.

2.2.2 Juvenile stage one (J1)

At this stage the juveniles molts within the egg and it's formed at the end of embryogenesis (Massawe, 2010). Juvenile continues to develop and it spontaneously molts into J2.

2.2.3 Juvenile stage two (J2)

Juveniles are very small about 0.5mm long. It is in vermiform and develops to become sedimentary, this is the most destructive stage (Haque 2012).During this stage the juvenile uses

food reserves in the intestines in the form of protein and lipids for survival (Galleti *et al*., 2008) until it finds a suitable host, it feeds ectoparasitically on the root tissue. Stage 2 juveniles are attracted by root exudates and the move towards the root tip for where it penetrates into the plant roots. RKN uses the stylet to penetrate the root cells and injects growth regulating substances into the cells through the stylet (Ferraz and Brown, 2002).

Giant cells are formed these are transformed parenchyma cells within the central and vascular cylinder that are essential for root-knot nematode growth and development. Surrounding root tissue develops the nuclei of these elongated cells, multiply while the cytoplasm becomes dense and cell wall thickens forming root galls in which the juvenile are embedded in. J2 then molts to juvenile stage 3 (J3).

2.2.4 Juvenile stage 3 (J3) and 4 (J4)

Juveniles at this stage are sedimentary inside the roots, sausage shaped (swollen), microscopic in size (Siddiqi and Shaukat 2004) and also lack the stylet.

2.2.5 Adult

The last stage is the adult stage identified by a pear shaped, white females (Bernard *et al*., 2017) with mature females measuring about 22-37nm in diameter and males which are wormlike, small and thin measuring 887-1268nm in diameter. Males are not necessarily required for reproduction as *Meloidogyne* spp reproduce by parthenogenesis (Massawe, 2010). At adult stage feeding resumes and development of reproductive system begins. Mature female lays about 30-50eggs per day thus producing more than 8 generations per year. Lifecycle ends with the release of eggs which are in the gelatinous egg matrix (Bernard *et al*., 2017). Hatching of eggs is dependent on

the temperature and host plant and it takes about 21-28days to complete lifecycle at 30ºC *(*Karseen and Moens, 2006).

2.3 Effects of root-knot nematodes

Plant parasitic nematodes are biotrophic parasites which obtain nutrients from the cytoplasm of living roots, stems and leaf cells for development, growth and survival (Luc *et al*., 2005). Nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants (Davis *et al*., 2004). Depending on the species, they feed from the cytoplasm of unmodified living plant cells or have evolved to modify root cells into elaborate feeding cells as in RKN (Lee, 2002; Luc *et al*., 2005). The nematodes use their stylet to pierce and penetrate the cell wall of a plant where they inject gland secretions through the stylet orifice into the cell and then withdraw and ingest nutrients from the cytoplasm (Bilgrami and Gaugler, 2004).

Nematodes that enter the root tissue also use their stylet to pierce openings and/or inject secretions to dissolve (intracellular migration) or weaken (intercellular migration) the cell wall or middle lamella (Lee, 2002; Bilgrami and Gaugler, 2004). Generally, all plant parasitic nematodes damage plants by direct mechanical injury using the stylet to penetrate and/or secrete enzymes into the plant cells while it is feeding (Gheysen and Jones, 2006). The physical presence of endo-parasitic nematodes in the host also affects the functioning of the host. As a result of nematode feeding, the architecture and extent of the root system is altered, so that it is less efficient at taking up nutrients and water from soil (Lee, 2002). The extent of nematode damage depends to a large extent on the inoculum density (level of infestation). Low or moderate numbers of nematodes may not cause much injury but large numbers severely damage or kill their hosts (Luc *et al*., 2005).

2.4 Damage symptoms

In most cases root-knot nematodes do not produce specific above ground symptoms, affected plants exhibit stunted growth .In severe cases there is wilting of the affected plants this occurs late in the season when there is build-up of nematodes (Hay *et al*., 2014). Plants also show signs of nutrient deficiency with discoloration of leaves (chlorosis). During the reproductive stage plants undergo premature fruit drop and flower abortion reducing crop yields.

Root-knot nematode underground symptoms are quite distinctive, there is formation of root galls usually 1-10 mm in diameter. Gall formation starts soon after invasion of the roots by stage 2 juveniles (J2). Accoding to (Hay *et al*., 2014), as the crop matures galls continue to increase in size and size of galls differ with infected plant species. Plants roots become swollen and deformed or distorted, these ultimately cause poor root development and development of some fibrous roots or hairy roots. (Bernard *et al*., 2017) recorded abnormal flower production in sweet potatoes which contributed to yields.

2.5 Economic importance

Root-knot nematodes reduce plant vigour, quality and quantity causing annual yield losses of more than $78billion worldwide (Tariq-khan *et al*., 2017). They have been noted to attack more than 2000 species of plants and almost all cultivated plants e.g. ornamentals, vegetables (Muzhandu *et al.,* 2014). Plant parasitic nematodes also predispose plants to secondary infections by pathogens. Yield losses can be caused by bacteria and fungal interactions with nematodes causing disease complexes which result in devastating yield losses of about 80% in tobacco. In tomatoes the interaction between *Fusarium oxysporum lycopersia* and nematodes causes severe wilting disease of tomatoes (Hay, 2014).

2.6 Root-knot nematodes control methods

2.6.1 Chemical control

Control by nematicides is the most rapid and effective way of protecting plants against nematodes and most effective (Karavina and Mandumbu, 2012). Root-knot nematode are polyphagus so chemical is likely the main method of control. Nematicides have been used extensively since the 1900‟s (Ferraz & Brown, 2002) as the major control strategy to reduce nematode numbers in high value crops such as vegetables and a range of other crops. (Massawe, 2010) classified nematicides into non-fumigants and fumigants, fumigants are rated to be 50-90% effective in controlling nematodes examples ethylene dibromide, methyl bromide and methan sodium. In Zimbabwe farmers use non-fumigants nematicides such as (Nemacur) Fenamiphos®40Ec, carbonfuran and (aldicarb) temick®SG. These non-fumigants are less phytotoxic compared to fumigants, do not have broad spectrum activity and have low persistence in the soils. Fumigants have a broader spectrum of activity controlling pests (beneficial insect's pests), fungal disease and weeds, they also cause food contamination and environmental pollution, high overhead costs and difficulties with application of these chemicals. These effects lead to the ban of some synthetic nematicides such as methyl bromide under the Montreal Protocol of 1997 (Karavina and Mandumbu, 2012)

2.6.2 Cultural control

The high costs and potential health and environmental hazards of agricultural chemicals are turning nematode control options towards non-chemical or cultural methods. The following activities are used to interfere with nematodes survival and reproduction (Karavina and

Mandumbu, 2012). They cannot fully eradicate root-knot nematodes but can used in managing them they are used in combinations.

2.6.2.1. Crop Rotation

It's the practise of growing different crops in succession on the same piece of land. Crop rotation is more suited to low-value annual and short-term perennial crops and very effective against nematode species with narrow host ranges (Kleynhans *et al*., 1996) .Closely related crops are likely to support the same nematode species so planting crops with different susceptibility will help in reducing RKN damage. Polyphagus species wide a wide host range such as RKN limits the effectiveness of this control method, however rotations are unsustainable to small holder farmers (Karavina and Mundumbu, 2012) but can be used by commercial farmers with vast land and time for crop rotations.

2.6.2.2. Fallowing

Method ensures that there is no continuous supply of food for the nematodes it works by starving them and exposing them to mechanical injury, desiccating effects of the sun, wind and climate (Karavina and Mandumbu, 2012). Removal of all plant roots and/or other nematode-infected plant tissue is essential as these may harbour endoparasitic species however bare fallowing exposes the soil to erosion this could be minimised by practising grass fallows *Eragrostis curvula, Panicum maximum a*nd *Cyanodon dactylon* have been used in root-knot nematodes control. Fallowing has to be economical and acceptable to the grower, therefore, it is most effective when other control techniques are used simultaneously (Kinloch and Dunavin, 1993), the longer the area fallowed the higher the efficacy of the method.

2.6.3 Physical methods

2.6.3.1 Heat treatment

Temperature above 45°C can be used to kill parasitic nematodes. Hot water treatment can be practised this involves immersion of the plant material in water heated to temperatures that will kill the nematodes without harming the planting material (Kleynhans *et al*, 1996:8). Heating the soil either with dry or steam heat has been used for many years in protected cultivation to manage root-knot nematodes, but the high cost of heating oil has limited its use drastically.

2.6.3.2 Solarisation

It's a form of soil pasteurisation or sterilising which involves trapping the suns energy (heat) under a plastic know as plastic mulching. It leads to the build-up of temperatures in the soil for about 30 days it's a cost-effective strategy to control root-knot nematodes and other soil borne diseases. Combinations of solarisation with chemicals (1.3D) and organic amendments can improve nematode suppression.

2.6.3.3 Soil steaming

Involves the use of high pressured steam directed to the soil, organisms are subjected to temperatures of about 70ºC which are lethally. According to Karavina and Mandumbu, (2012) this method is not sustainable as it kills all organisms' even beneficial organisms in the root zone, its slow and labour intensive.

2.6.4 Trap crops and Antagonistic plants

Trap cropping involves planting a crop which is highly susceptible to nematodes and fast growing on to an heavily nematode infested land, the crop is then uprooted and destroyed before

it reaches maturity and the nematodes have not completed their life cycle. Example include use of cowpea in controlling *Meloidogyne* spp (Karavina and Mandumbu, 2012).

Antagonics plants, these produce allelochemicals in their root which are toxic and act as repellent towards plant parasitic nematodes. Examples of these include Asparagus roots which produce asparaguric acid glycoside that is toxic to most parasitic nematodes. Other allelochemicals such as alkaloids terthienyl, hyosine and ricinini produced by *Tagetes*, *Datura stramonium* and *Ricinus communis* respectively have been noted to induce premature nematode hatching, block mitosis and reduce galling intensity on roots (Karavina and Mandumbu, 2012).

2.6.5 Resistant cultivars

Resistance of plant parasitic nematodes has been described as a set of characteristics of the host plant which act more or less to the detriment of plant parasites such resistance is recognised as either resistance to the nematode and its development or reproduction or to resistance to the disease caused by the nematodes. According to Karavina and Mandumbu, (2012) most of the plant resistance genes are more effective against sedimentary endoparasitic nematodes species (*Meloidogyne*, *Globodera* species) compared to migratory nematodes (*Xiphinema*, *Trichodorus*). Resistant crops provide useful, effective and economical method for managing nematodes in both high- and low-cash value cropping systems (Chitwood, 2002).

2.6.6 Biological control

Use of biological control agents (BCA), it's considered most economic and environmentally safe control method. Fungi such as *Trichoderma harzianum* and *Hirsutellar hossiliensis* have being noted to infect nematode eggs and juveniles (Sahebani and Hadavi 2008).some organisms such as rhizosphere bacteria can be applied as seed treatment but such applications tend to provide

short term control and are useful in reducing the invasion of roots by nematodes. Biological control agents' efficacy is dependent on pest densities, they are only effective against specific nematode pests and they are also slow acting.

2.6.7 Bio-fumigation

The term 'bio-fumigation' was originally coined by J.A. Kirkegaard to describe the process of growing, macerating / incorporating certain *Brassica* or related species into the soil, leading to the release of isothiocyanate compounds (ITCs) through the hydrolysis of glucosinolate (GLS) compounds contained in the plant tissues (Kirkegaard *et al*., 1993). This result in a suppressive effect on a range of soil borne pests especially nematodes and diseases. Other researchers such as (Halbrendt, 1996; Kirkegaard and Sarwar, 1998) defined bio-fumigation as a process that occurs when volatile compounds with pesticidal properties are released during decomposition of plant materials or animal products of which Cruciferous plants belonging to *Brassica* spp contains these glucosinolate compounds as toxic products (e.g. thiocyanate, isothiocyanate) (Brown and Morra, 1996). These are produced when plant cells are damaged by crushing or chopping. After that, compounds interact with an enzyme called myrosinase (MYR) in the presence of water and produce D-glucose, isothiocyanate (bio- fumigant) and nitrite (Youssef, 2015).

Since then, the term 'bio-fumigation' has been used rather loosely and incorrectly in some contexts, to describe any beneficial effects derived from the use of green manures, organic amendments and composts. In this mini-paper, bio-fumigation is considered in its strictest sense as referring to the use of glucosinolate-containing plant material with the intention of enabling ITC-mediated pest and disease suppression. Bio-fumigation could be considered as a 'natural' alternative to chemical fumigation and there is an analogy with the use of metam sodium which releases methyl-ITC, to control a variety of soil borne diseases.

2.6.7.1 Glucosinolate / isothiocyanate and chemical effects

Many cruciferous species produce significant levels of glucosinolates (GLSs), which are held in plant cells separately from the enzyme MYR (Manici *et al*., 1997). However, when plant cells are ruptured the GLSs and MYR come into contact and are hydrolysed in the presence of water to release various products, including ITCs (Vig *et al*., 2009). ITCs have a wide range of biocidal characteristics and are acutely toxic to a variety of pests and pathogens. GLSs are β-thioglucoside N-hydroxysulfates, with a side group (R) and a sulphur-linked β-d-glucopyranose moiety (Fahey *et al*., 2001) and are classified as aliphatic, aromatic or indole GLSs according to the type of side chain. The R group is retained in the ITCs and influences its biological activity. Commonly used bio-fumigant plants which include brown mustards, white mustards, radishes and rocket species contain different GLSs hence resulting in different ITCs being released. According to Salem, (2012) mustards produce a high content of oxygenated compounds which are characterised by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure causing mortality. Although some bio-fumigants have a dominant GLS, others may contain a mixture. Different cultivars or plant parts may also contain different amounts or profiles of GLSs (Karavina and Mandumbu, 2012)

2.6.8 Plants containing glucosinolates

The Family Brassicaceae contains more than 350 genera with 3 000 species of which many are known to contain GLS. However, GLSs are not confined to brassicas alone. At least 500 species of non-*brassica* dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GLSs (Fahey *et al*., 2001). Each of the GLSs has its own chemical property

and can be placed in one of three different classes, namely aliphatic, aromatic or indole forms (Zasada & Ferris, 2004). Most GLS-containing genera, however, are clustered within the Brassicaceae, Capparaceae and Caricaceae families. The GLS concentration in the cells of the various plants in the families differs substantially. Therefore, it is crucial to identify species that will be effective in supressing soil-borne pests and diseases, including nematodes.

2.6.8.1 Brassica juncea

Brassica juncea (L) or Indian mustard it belongs to the mustard family (Brassicaceae) and is known to possess GLSs in its vacuoles. Centres of Diversity are in African and Eurosiberian regions. Sinigrin, which is a glucosinolate found in *B. juncea*, which produces allyl-isothiocyanate or 2- propenly ITC, glucose, and potassium bisulfate when hydrolysed by myrosinase. Other ITCs in *Brassica juncea* include 3-butenyl, 4-pentenyl and 2-phenyethylin, Benzyl (Karavina and Mandumbu, 2012) these ITCs also affect development , hatching and reproduction of RKNs. 2-propenly ITC have been reported to bind to proteins and cause cell epigenesis and apoptosis (Ferguson, 2009) thereby reducing nematode populations. Sinigrin glucosinolate in *Brassica* spp is said to be toxic to some insect larvae but harmless to others (Brown and Morra, 2005). In potato production *B. juncea* applied as green manure recorded a 91-95% mortality in encysted eggs of RKN.

2.6.8.2 Brassica carinata

Brassica carinata belongs to the Brassicaceae family. It is a traditional African vegetable commonly known as Ethiopian mustard. It is believed to have originated from the Ethiopian highlands and its cultivation is thought to have started about 4000 years B.C. (Alemayehu and Becker, 2002). *B. carinata* has been widely used as a source of bio-fumigation due to its high

concentration of GLSs. It process 2-propenly as the primary isothiocynates which have high toxicity activity against nematodes.

2.6.8.3 **Purple and green stem *Cleome gyanandra***

Cleome gynandra it's an herbaceous annual or perennial plant / shrub commonly known as Cat's whiskers, it belongs to the Caperraceae family. It's said to have originated in tropical Africa and South East Asia and it spread throughout the world in tropical and subtropical regions it has 150-200 species of which 50 of these are indigenous to Africa (Silue *et al.*, 2009). Natural habitat of *C. gynandra* is on wastelands and arable land alongside annual species as well as on grasslands. (Naidu *et al*., 1980). Spider plants contains glucosinolates, including methyl glucosinolate, cleomin, and glucocapparin. Hydrolysis of these elements yields methyl isothiocyanates which is a strong antimicrobial compound known to possess insecticidal properties, along with phenolic compounds .According to Nyalala, (2013) Homogenized leaves of *Cleome gynandra* emit significant quantities of methyl-isothiocyanate, proply-isothiocyanate, butyl-isothiocyanate and isobutyl-isothiocyanate plus a number of aldehydes, terpenes, alcohols, acetates and ketones.

Table 1: Bio-fumigant plants and their respective GLSs and ITCs

Common name	**Glucosinolates**	**ITCs**
Indian mustard (*Brassica juncea*)	Sinigrin	2-propenyl-ITC/Allyl-ITC
Ethiopian mustard (*Brassica carinata*)	Sinigrin	2-propenyl-ITC/Allyl-ITC
Cat´s Whiskers green (*Cleome gynandra*)	glucocapparin	methyl isothiocynates
Cat´s Whiskers purple (*Cleome gynandra)*	glucocapparin	methyl isothiocynates

Adapted from Gimsing and Kirkegaard, 2009)

Although ITCs have generally been the focus of bio-fumigation-related research and are considered the most bioactive of the hydrolysis products, other compounds such as non-glucosinolate sulphur-containing compounds, fatty acids, nitriles and ionic thiocyanates may also affect pest and pathogen populations (Matthiessen & Kirkegaard, 2006) and may explain why some low GLS *Brassica* crops have been shown to have suppressive activity against soil-borne pathogens.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Horticulture Research Centre in Mashonaland East Province which is in Zimbabwe agro-ecological region IIa located at $18^0$11'S and $31^0$28'E at an elevation of 1630m above sea level. Area receives an average maximum temperature of 30ºC and average min temperature of 22ºC. Experiment was carried out under laboratory conditions (*In vitro* experiment).

3.2 Experimental design

The experiment was laid out as a 4×3 plus 1 (positive control) factorial in a Compete Randomised Design (CRD) with 13 treatments replicated 4times to give a total of 52 experimental units, Factor A was type of bio-fumigant with 4 levels that is, *Brassica carinata*, *Brassica juncea*, purple stem *Cleome gynandra* and green stem *Cleome gynandra* and Factor B was concentration or rate with 3 levels that is 3, 5, 10 grams , nemacur at (0.5ml/10mls) rate were used as the positive control.

Table 2: Treatment table

Treatment	Treatments description g/100 nematodes
1	Purple *Cleome gynandra* 10g
2	Purple *Cleome gynandra* 5g
3	Purple *Cleome gynandra* 3g
4	Green *Cleome gynandra* 10g
5	Green *Cleome gynandra* 5g
6	Green *Cleome gynandra* 3g
7	*Brassica juncea* 10g
8	*Brassica juncea* 5g
9	*Brassica juncea* 3g
10	*Brassica carinata* 10g
11	*Brassica carinata* 5g
12	*Brassica carinata* 3g
13	Nemacur®400EC(positivecontrol) 0.5mils/10mls of water

3.3 Experimental procedure

3.3.1 Nematode preparation

Pure culture nematodes of *Meloidogyne javanica* were collected from Tobacco Research Board (TRB) in Harare at juvenile stage 2(J2) in solution.

3.3.2 Serial dilutions of the nematode solution

Serial dilutions were done by collecting 10 mls of the pure culture solution using a syringe and place the solution in to an empty 350 mls glass jar, 10mls of tap water was collected using a

springe and mixed with the pure nematode culture solution and a sample of the mixture was placed on a counting chamber and observed the concentration of the nematodes under the Labomed -310 stereo-microscope at X100 magnification. The process of adding 10 mls of water was repeated until we got 100 nematodes (J2) in 1mls pure solution.

3.4 Treatment preparation and administration

3.4.1 Collection of plants

Plants were collected by cutting the plants at 2cm above the soil using a knife and washed under running water and left to sun dry. The whole plant was chopped into small pieces using a knife and preserved in the refrigerator at a temperature of -5 ºC, each bio-fumigant packed in different plastic bags, after 1 week they were macerated using the Vitamix industrial blender (pro series 750).

The macerated bio-fumigants were taken out of the refrigerator and placed in 4 different plants that is each botanical in its own plate. Weighing of the bio-fumigants was done using 3000×0.01g analytical balance (scale) to get the different rates. Sterilised sand soil was mixed with different bio-fumigants at a rate of 10grams/10 grams of bio-fumigant. The mixture was packed in perforated black plastics measuring 12×8cm.

100 root-knot nematodes (J2) placed in a 500mls empty glass jar and the bio-fumigants in the plastics were placed on the mouth of the bottle before sealing with paraflim. Nemacur 400EC chemical was also added in the glass jars with nematodes as the positive control.

3.5 Data collection

3.5.1 Root-knot nematode mortality

Data on nematode mortality was measured at 21 days after establishment of the experiment.10 mls of the liquid with nematode exposed to treatments were collected and placed on a counting chamber and viewed on a Labomed -310 stereomicroscope at ×100 magnification. Dead nematodes were noted by counting non-moving or vibrating nematodes.

$$\text{Percentage mortality} = \frac{\text{total number of nematodes} - \text{total number of dead nematodes}}{\text{Total number of nematodes}} \times 100$$

3.5.2 Root-knot nematode egg count

Data on nematode mortality was measured at 21 days after establishment of the experiment. 10 mls of the liquid with nematode exposed to treatments were collected and placed on a counting chamber and viewed on Labomed -310 stereo microscope at x100 magnification. Root-knot nematode eggs were noted by using morphology characteristics (roundness and size).

3.6 Data analysis

Data was transformed using the square root transformation to normalize data count. The transformed data was subjected to the analysis of variance using Genstat 18th Edition. Treatment means were separated using Least Significant Difference at 5% significance level.

3.7 Characterisation of bio-fumigant plants used

Cleome gynadra used in this study contained total isothiocynates content of 19 and 7 μmol g^{-1} for purple *C. gynandra* accessions and green *C. gynadra* respectively. *Brassica juncea* and *Brassica carinata* contained isothiocynates content of 109 and 74 7 μmol g^{-1} respectively with the primary isothiocynates: Methyl-ITC for *Cleome* spp and 2 propenly –ITC for *Brassicas*.

Table 3 : Bio-fumigants glucosinolates profiling

Bio-fumigants	Primary isothiocynates	Amounts per gram dried green μmol g^{-1}
Purple stem *Cleome gynandra*	methyl- ITC	19
Green stem *Cleome gynandra*	methyl- ITC	7
Brassica juncea	2propenly-ITC	109.9
Brassica carinata	2propenly-ITC	74

CHAPTER 4

RESULTS

4.1 Effects of different bio-fumigants and rates on juvenile (J2) nematode mortality

There was an interaction between bio-fumigant and rates ($p \leq 0.04$) on nematode mortality .There was a general increase in nematode mortality with increase in concentration of botanicals. When all bio-fumigants were compared *Brassica juncea* recorded the highest nematode (J2) mortality at 10 grams followed by *B carinata*, purple and green stem *C gynandra*. Green *Cleome gynandra* at 3grams recorded the least mortality. Applying 10 and 5 grams of green *C gynandra* recorded percentage mortality showed no statistical difference. *Brassica juncea* at 10grams recorded mortality that was not statistically different from mortality recorded where nemacur was applied. When bio-fumigants were compared by plant species, the *Brassicas* performed better than *Cleome gynandra* species recording better mortalities at all application rates.

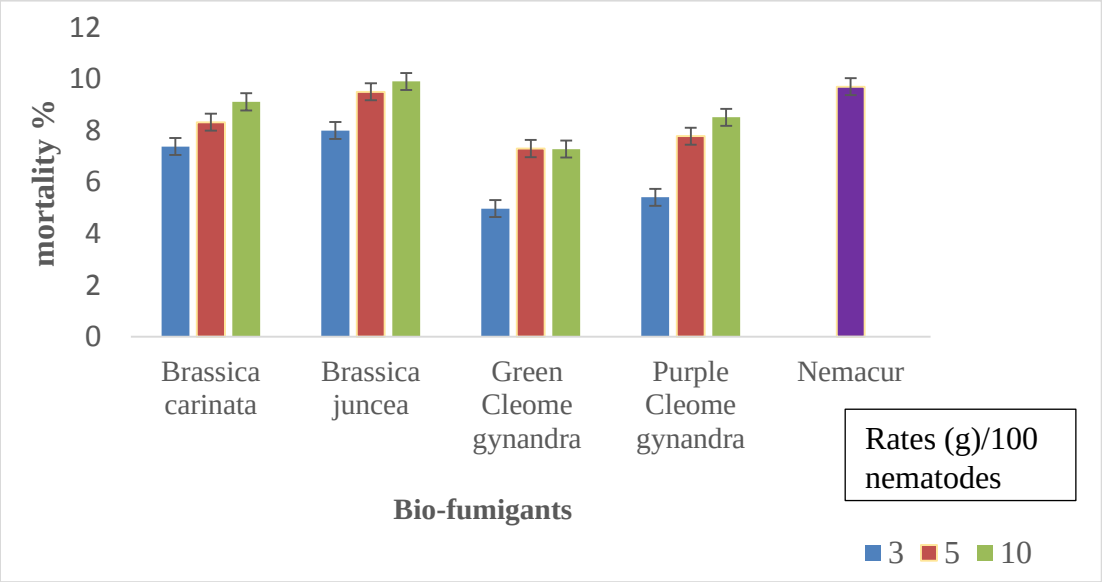

Figure 3: Effects of different bio fumigant rates on Root-knot nematode mortality.

4.2 Effects of different bio-fumigants on number of nematode eggs

There was no interaction between bio-fumigant type and rates on number of nematodes eggs. However, there was significant effect on the efficacy of different bio-fumigants on number of nematode eggs ($p \leq 0.01$). *Brassica juncea* recorded the least number of egg followed by *Brassica carinata*, purple *Cleome gynandra* and green *Cleome gynandra* respectively. When botanical species where compared *Brassicas* performed better than Cleome species (*Caperraceae species*). The number of nematode eggs recorded in *Brassica carinata* and *B .juncea* were statistically different. The same also apply to the number of nematode eggs recoded in purple and green stem *Cleome gynandra.*

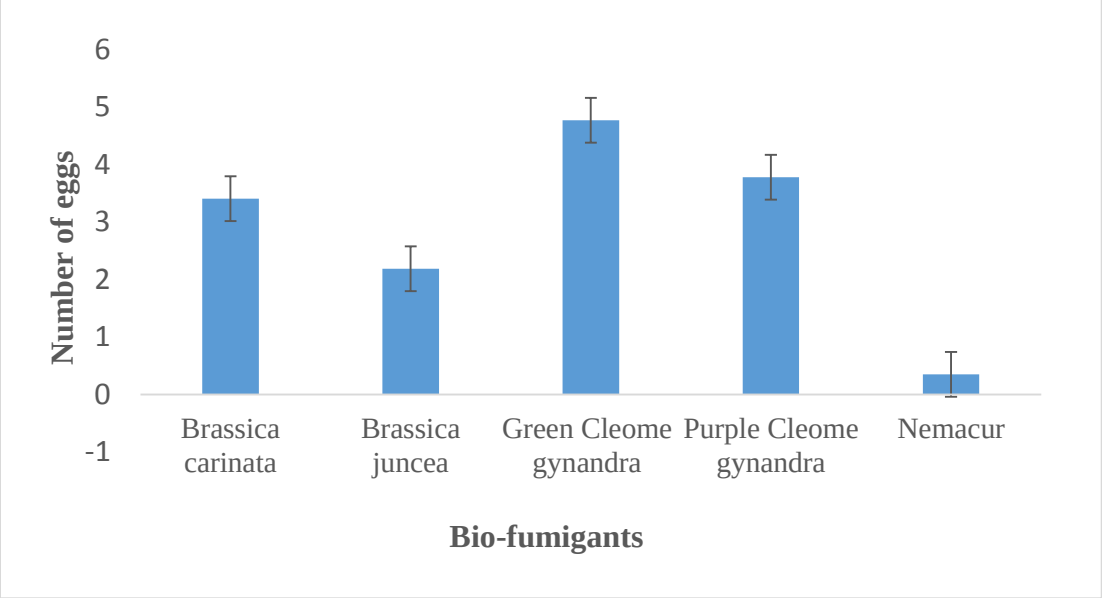

Figure 4: Effects of bio-fumigants on number of nematode eggs

4.3 Effects of different bio-fumigant rates on number of nematodes

Results show that there was no interaction between bio-fumigant type and rates on number of nematodes eggs. Results also show that there is significant difference ($p \leq 0.03$) on efficacy of different rates on controlling Root-knot nematode eggs .There was a general decrease in number of eggs with increase in rates.10grams recorded the least number of eggs followed by 5grams then lastly 3grams which recorded the highest egg count. There was no statistical difference on number of eggs recorded where 10grams, 5 grams and 3grams of the bio-fumigants was applied.

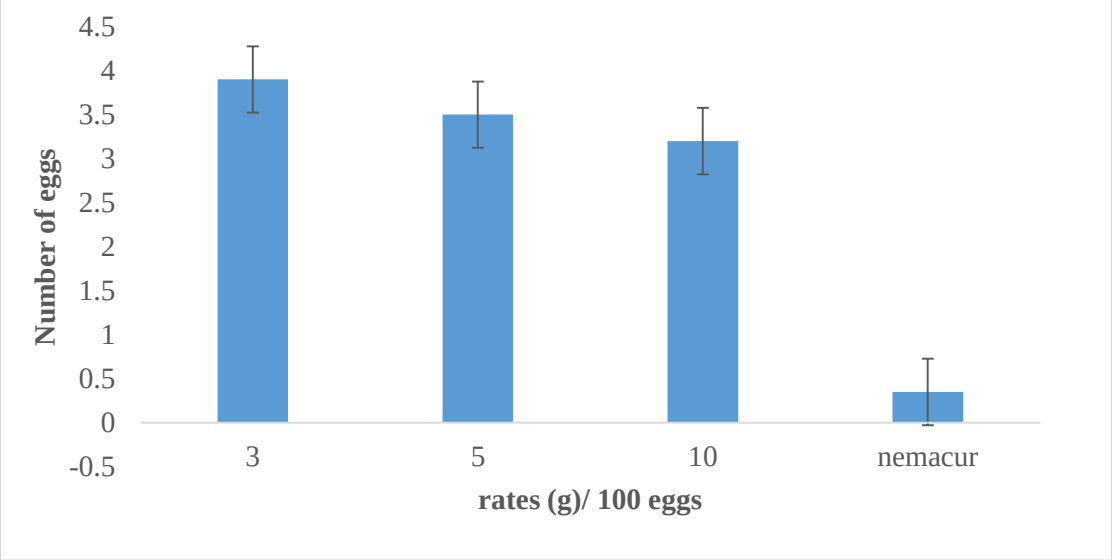

Figure 5: Effects of different rates on number of eggs

CHAPTER 5

DISCUSSION

5.1 Influence of different bio fumigants and rates on juvenile mortality

From the results there was an interaction between bio-fumigant and rates on juvenile mortality**,** when all bio-fumigants were compared *Brassica juncea* recorded the highest nematode (J2) mortality at 10 grams followed by *Brassica carinata*, purple and green stem. *Brassica juncea* contains volatile compounds such as 2 propenly isothiocyanates which reacts with biological nucleophiles essential for the nematode, mainly thiol and amine groups of various enzymes which become irreversibly alkylated (Gimsing and Kirkegaard, 2006), this could have contributed to juvenile mortality recorded. According to isothiocynates analysis by Buena *et al*., (2007)*Brassica juncea* also contains epthionitriles, Benzyl, Isoproply, 1-napthyl 2 phenylethly and oxazolidinethione which were noted to suppress nematode populations by degrading and denaturing of proteins this could have contributed to the increased juvenile mortality recorded where *Brassica juncea* was used. Results also indicated that when Brassica spp were compared, *Brassica juncea* performed better than *Brassica carinata*, Even though the 2 contain the same ITCs that is 2 propenly-ITC the concentration recorded in *Brassica juncea* was high 109.9 μmol g^{-1} than in *Brassica carinata* 74 μmol g^{-1} this is probably why *Brassica juncea* recoded the higher mortality.

Green *Cleome gynandra* was the least performer at all rates in terms of mortality.*Cleome gynandra* contains methyl glucosinolate, cleomin and glucocapparin which give rise to methyl isothiocyanates when hydrolysed (Silué, 2009), However, according to Nyalala *et al*., (2013) methyl isothiocynates is highly volatile and its lost during harvesting and preparation thereby

reducing their effectiveness. According to Matthiessen and Kirkegaard, (2000) methyl ITC is known to possess a high diffusability rate compared to 2 propenly ITC and it also decompose quickly into inactive harmless compounds this could have reduced its efficacy in controlling rootknot nematodes.

The *Brassica* species (*B. carinata* and *B. juncea)* outperformed purple and green stem *Cleome gynandra* species at all rates with respect to juvenile mortality. Zasada and Ferri, (2003) noted that *Brassicas* contain glycosidic compounds whose enzymatic hydrolysis degradation products (isothiocyanates and nitriles) are well-known for their high cytotoxic activity and its aliphatic short chained ITCs which are more efficient and increases volatility when compared to long chained aromatic ITCs from *Cleome* species (Lazzeri *et al*., 2004; Sanchi *et al*., 2004) this could have increased efficacy of Brassica species.

There was a general increase in nematode mortality with increase in concentration of botanicals with 10g producing highest mortality rate which was significantly similar to control. This might be due to high amounts of biomass producing maximum level of GLS which renders effective control. This is supported by (Kirkegaard and Sarwar, 1998) who suggested that up to 10% w/w fresh biomass is required to maximize pathogen suppression.

5.2 Effects of different bio-fumigants on number of nematodes eggs

Results show that there was no interaction between bio-fumigant type and rates on number of nematodes eggs. However, there was significant effect on the efficacy of different bio-fumigants on number of nematode eggs recorded**.** Results of this study demonstrated that bio-fumigation with *Brassica juncea* induced high inhibition activity against *Meloidogyne* spp as proved by the high significant reduction of the number of eggs compared to other species.

There is a relationship between mortality percentage and number of eggs recorded. *Brassica juncea* recorded the highest mortality percentage. This could have reduced the number of nematodes that would have reproduced eggs therefore reducing the number of eggs .Green *Cleome gynandra* recorded the least juvenile mortality therefore more nematodes reproduced hence an increase in number of eggs recorded. (BITC) Benzyl isothiocynates in *Brassica juncea* has being found to suppress nematode reproduction of *M. javanica* species to about 98% suppression (Masler *et al*., 2010). BITC affects embryo development as well as nematode development this could have contributed to high mortality recorded on nematodes exposed to *Brassica juncea*.

Within the *Brassicas*, the *Brassica juncea* was highest in reducing the number of eggs to lowest level which was comparable to control in its efficacy. This is due to (*Brassica juncea*) having highest levels (109.9 μmol g^{-1}*)* of 2-propenly ITC which is said to reduce *M. javanica* populations and enhancing egg hatch inhibition (Davis, 2004). It is these isothiocyanates that give *B. juncea* its bio fumigation power.

5.3 Effects of different rates on nematode number of eggs

Results show that there was no interaction between bio-fumigant type and rates on number of nematodes eggs, However there is significant difference on efficacy of different rates on controlling root-knot nematode eggs. In terms of rates used, the least amount of 3g produced higher number of nematodes eggs which indicates low dosage rate which is ineffective as a result of low amount of isothiocyanates being generated compared to higher rates of 5g and 10g which render effective control of reducing egg number as a result of higher level of isothiocyanates contained since it is the level of biomass and amount of glucosinolates as factors that are fundamental to the success of bio-fumigation this results are in agreement with (Zasada and Ferri

2003) who also recorded that (8.5 w/w) of *Brassica juncea* amendment reduced egg hatching by at least 72% compared to low levels (2 w/w) where there was no nematode suppression or increased egg hatching.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the results it is concluded that *Brassica juncea* is the most effective botanical in suppressing *M. javanica* populations. *B. juncea* was the best performer recording the highest mortality and reduced number of nematode eggs compared to other botanicals used.

Brassica juncea increased juvenile mortality and reduced number of eggs recorded, it performed almost the same as the chemical Nemacur 400Ec.

When bio-fumigants were compared *Brassica juncea* at all rates was the most effective followed by *Brassica carinata*, purple stem *Cleome gynandra* and the least effective was green stem *Cleome gynadra* recording the lowest mortality percentage and recorded the highest egg count.

Results indicated that efficacy of bio-fumigants increased with increase in concentrations where *Brassica juncea* at 10 grams recorded the highest mortality compared to *Brassica juncea* at 5 grams which recorded low mortality.

6.2 Recommendations

Basing from the results farmers are recommended to apply *Brassica juncea* to control nematodes. Other biofumigants used in the study (*Cleome species* and *B. carinata*) can be used at higher rates to effectively control root-knot nematodes. However, other researchers can increase rates to see if it can further improve nematode suppression. Further studies can be done on other botanicals that were not used in this experiment to be evaluated for biocidal effects on RKNs.

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APPENDICES

A 1: ANOVA for the effects of different bio-fumigants and rates on juvenile mortality

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fumigant	1	13.4944	13.4944	61.92	<.001
Fumigant. Type	3	47.9324	15.9775	73.32	<.001
Fumigant. Amount_ Grams	2	44.9079	22.4540	103.03	<.001
Fumigant.Type.AmountGrams	6	4.0214	0.6702	3.08	0.015
Residual	39	8.4992	0.2179		
Total	51	118.8553			

A 2 : ANOVA for effects of bio-fumigants and rates on number of root-knot eggs

Source of variation	d.f	s.s	m.s	v.r	F pr.
Fumigant	1	37.3691	37.3691	81.27	<.001
Fumigant. Type	3	40.9551	13.6517	29.69	<.001
Fumigant. Amount_ Grams	2	3.4648	1.7324	3.77	0.032
Fumigant. Type. Amount Grams	6	5.3703	0.8950	1.95	0.097
Residual	39	17.9336	0.4598		
Total	51	105.0929			