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EFFECTS OF TOBACCO (Nicotiana Tabacum) ROTATION AND FUMIGATION ON THE TEMPORAL AND SPATIAL DISTRIBUTION OF ROOTS-KNOT NEMATODES (MELOIDOGYNE SSP)

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ABSTRACT

Root-knot nematodes cause significant yield losses in tobacco (*Nicotiana tabacum*). Their management is essential for the grower to maximize production. Nematodes migrate up and down the soil profile in response to host availability. The objective of the study was to evaluate the effect of tobacco rotations on the spatial and temporal distribution of nematodes. Sampling was done every month from September 2010 to April 2011. The experimental design was a split-plot in a randomized complete block design. The main plot factor was fumigation and subplot factor was rotation. The rotations were sugar beans- tobacco, sunn hemp- tobacco, winter katambora - tobacco (all winter crops) and a fallow –tobacco. In each of the 8 treatments sampling was done at three different positions. At each sampling position sampling was done to a depth of 45cm at intervals of, (0-15 cm), (15-30 cm), (30-45 cm). Data was transformed and analysis of variance ANOVA was done using the statistical package Genstat (Version 7.22). No interactions were found between fumigation and rotations in all three depths ($p>0.05$). Significant differences ($p<0.05$) were recorded for the different rotations; In September 0-15cm the sugar beans rotation was significantly different. In March 15-30cm the winter katambora rotation was significantly different ($p<0.05$) and in January 30-45cm, the winter katambora rotation was significantly different ($p<0.05$). The spatial and temporal distribution of the nematodes in the three depths showed high nematode activity in the 0-15cm depth between March and April, while in the 15-30cm and 30-45cm depths, nematode populations were high between September and November. In conclusion sugar bean is not a suitable crop to include in tobacco rotations due to its susceptibility to nematodes. Winter katambora might not break the longevity of nematode eggs. In addition sampling should be done in April to obtain the correct nematode populations in the recommended sampling depth of 15cm-20cm. It is recommended that this experiment be repeated with a susceptible cultivar KM10 to obtain a good indication of the nematode distribution. It is also recommended that sunn hemp be used in tobacco rotations because it has the ability to suppress nematodes.

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DEDICATIONS

I dedicate this project to my family, my husband and our future children.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to study

Tobacco (*Nicotiana tabacum)* is a member of the solanaceae night shade family grown for its leaf, where the economic product (Nicotine), is extracted (Mazarura, 2005).Tobacco is an important commercial crop in view of revenue generation and export earnings and employment potential (Reddy, 2004). In Zimbabwe tobacco is one of major foreign currency earners contributing 25.5% to the GDP (Gono, 2011). Although tobacco output in Zimbabwe declined drastically between 2000 and 2008, the out-put has since increased (TIMB, 2011), with the 2010/11 tobacco growing season recording a total of 133million kg and the 2011/12 season expected to produce up to 150million kg by the end of the season (Financial gazette, 2012). Production of tobacco is affected by various pests and diseases that result in the lowering of its quality and quantity (Dimbi et al, 2010). The management of these pests and diseases is important for successful tobacco production to be achieved.

Pests and diseases affecting tobacco fall under at least eight broad categories namely: fungal diseases, bacterial diseases, mycoplasma diseases, viral diseases, diseases caused by flowering plants, malnutrition disease, some miscellaneous diseases and nematodes. Of the nematodes, root-knot nematodes cause significant yield losses in tobacco production, partly because their above ground symptoms are often confused with physiological damage and are similar to those of plants with damaged root systems (Moens et al, 2009). The root-knot nematodes (*Meloidogyne spp*) are worldwide in occurrence and were described by Lucas (1975) as a major problem in tobacco production throughout the world. Fortuna (2003) found that the root-knot nematodes accounted for 95 % of all nematode damage on tobacco. Globally, losses due to root-knot nematodes have been greatly under estimated yet they have accounted for approximately 5 % of all crop yield losses worldwide (Marais and Fourie,

2010). There is need for control and management of nematodes to avoid economic losses in tobacco production.

However one of the major challenges in identifying nematodes as the causal agent of crop damage is the fact that many of them do not produce highly diagnostic symptoms, which are specific and easy to identify (Coyne et-al, 2007). Various management practices have been used to manage nematodes and these can be categorized into five classes, namely chemical control, biological control, cultural control, varietal resistance and integrated pest management.

Over the years, emphasis had been on chemical control before the worldwide phase out of several important nematicides including methyl bromide and Aldicarb, which prompted need for alternative ecologically acceptable methods of managing nematodes (Duniway, 2002). Efforts have been made to manage nematodes using biological control agents in which the active principle is a nematode, bacteria or fungal agent which can either predate or parasitize natural enemies (pests and pathogens) Oudejans, 1991. Efforts have also been made to breed cultivars that are resistant to the root-knot nematodes. In addition, cultural control methods can be used as they cause the least damage to the environment. Of the cultural controls, rotations play a pivotal role in the management of root-knot nematodes however; these management practices work effectively when integrated together. A holistic approach to nematode control where safer nematicides and cultural control strategies are addressed in an integrated pest management approach (IPM), should thus be the ultimate goal.

1.2 Justification

Crop rotations have been found to be effective in nematode control but most small scale farmers are failing to implement them due to limited land and the fact that some of the rotations require more than a year to implement effectively. The integration of fumigation and rotations in this study seeks to evaluate the effectiveness of fumigation in tobacco

rotations on the distribution of nematodes in space and time. The worldwide phase out of nematicide like methyl bromide and Aldicarb as resulted in a great and pressing need to test and bring forth an alternative that will effectively control nematodes with as minimal detrimental effects as possible to the environment. The study seeks to develop an integrated approach to pest management by evaluating the effectiveness of both fumigation and tobacco rotations on the spatial and temporal distribution of nematodes. Nematodes migrate up and down the soil profile and this complicates management decisions.

The effectiveness of integrated pest management lies in the understanding of seasonal fluctuations in nematode populations and their distribution. The knowledge of the spatial and temporal distribution of nematodes enables sampling protocols to be evaluated for effective management strategies to be implemented. Nematodes found at deeper profiles complicate management decisions. Shallower placement of nematicides would result in the ridged area, which is virtually free of *M. javanica*, being fumigated, while the more heavily infested deeper profiles into which the downward growth of tobacco roots occurs, would escape treatment (Ferris, 1966). These populations later migrate and infest the crop after planting, provided the host and moisture conditions are conducive. At lower depths nematodes are buffered from surface temperatures and moisture extremes, which enhance their survival and reproduction (Marais et al, 2010). The understanding of the vertical distribution of nematodes is critical in nematode management.

The fact that the vertical migration of nematodes varies in response to host availability calls for the evaluation of the distribution of nematodes in the different tobacco rotations. The knowledge from this study would provide information to the grower on effect of different tobacco rotations on the distribution of nematodes. This would enable farmers to make effective decisions on the rotations that control nematodes to deeper depths or profiles for

effective management of nematodes. It would provide information on the best sampling time and depth for the farmer to sample to obtain a representative sample of nematodes in an area. It would also provide information on the effective rotation in managing nematodes to greater depths as well as provide information on the effectiveness of fumigation in tobacco rotations.

1.3 Objectives

1.3.1 Overall Objective

- To evaluate the effect of tobacco rotations and fumigation on the spatial and temporal distribution of nematodes.

1.3.2 Specific objectives

- To determine the effects of different tobacco rotations on the vertical distribution of nematodes.
- To determine the temporal distribution of nematodes under different tobacco rotations and fumigation.

1.3.3 Hypotheses

- There is no significant difference in spatial distribution of nematodes due to different tobacco rotations.
- There is no significant difference in temporal distribution of nematodes under different tobacco rotations and fumigation.

CHAPTER TWO

2.1 Tobacco Botany and importance

Tobacco is a member of the *Solanaceae* night shade family (Hartwig and Amon, 2002), which also includes many crop species such as tomatoes, potatoes and peppers. The tobacco plant is a fairly diminutive plant in the biological family known as the *Solanaceae*. The family includes over 60 spp of the genus *Nicotiana.* Of these only one species *Nicotiana tabacum* retains major commercial importance (Wang et al, 2003). The tobacco plant is a rank-growing annual crop retarded root development compared to the canopy (Reddy, 2004).It is grown for its leaf where nicotine, the major economic product is extracted (Mazarura, 2004).The tobacco industry is an important segment of world business. Flue cured tobacco is a major product of Zimbabwe and provides the backbone of her agricultural economy (World Resource Institute, 2002).

2.2 Diseases and pests of tobacco

The extraction of food from one organism by another organism inevitably entails some disturbance of the host integrity. The disturbance is usually something foreign and unusual that changes the existing order of things and upsets the stability of the host. If the disturbance persists it may produce a series of events that create different and more drastic disturbances which we call the symptoms of disease (Hutchinson et al, 1999).

Diseases and pests of tobacco can be divided into eight broad groups which are nematode, fungal, bacterial, viral, those caused by flowering plants, malnutrition and miscellaneous diseases. Some examples of some common diseases affecting tobacco the viral diseases are bushy top and PVY these pathogens cause a significant amount of yield loss in association with some fungal diseases. Common diseases include *Alternaria, Pythium, Rhizoctonia* leaf spot, bacterial or Granville wilt, tobacco mosaic virus (TMV), black shank and root knot

nematodes Insects also cause significant yield losses especially the aphids (Dimbi et al, 2010). Of the nematode species, the root-knot nematode were found to cause significant yield losses in tobacco soils in Zimbabwe (York and Nyamadzawo, 1999)

2.3 Description of the root-knot nematodes *Meloidogyne spp*

The root- knot nematode is probably the major disease problem in tobacco production throughout the world. The root-knot nematodes are sedentary, obligate endo-parasites that have evolved specialized and complex relationships with their hosts (Marshal, 2002). In many of the warmer growing regions the crop cannot be grown profitably without the use of successful control measures. This was due to a combination of factors including the occurrence of several dry, hot years in succession, the use of new cultivars which were not as tolerant to root – knot as earlier assumed (Lucas, 1975). Full recognition of the magnitude of crop damage resulting from nematode attack and the development of effective control measures has occurred in Zimbabwe since as early as 1938. It became strikingly evident that root- knot often lead to increased susceptibility of crops to other diseases caused by facultative microbes (Walker ea-al, 2002) causing excessive losses.

2.3.1 Distribution of the root knot nematodes

The root–knot nematodes (*Meloidogyne spp*) is a cosmopolitan genus found in all six biogeographically regions of the world (Kleynhans 1991). This disease is particularly severe on light sandy or sandy loam soils in warm and relatively cold areas climates and observations showed that the nematode can survive temperatures as low as -15°C. Root – knot causes less damage in cooler climates than warmer climates, presumably because the shorter growing season and lower soil temperatures reduce nematode activity, feeding and reproduction. Accurately assessing the potential for plant-parasitic nematodes to cause crop damage depends on accurate population estimates which are based on the knowledge of the horizontal and vertical distribution of the population (Fordge et-al, 1998)

2.3.2 Identification and Morphology of the root knot nematodes

A distinct feature of this genus is the occurrence of sexual dimorphism. The female body is pear shaped or ovoid and sedentary opposed to vermiform, mobile males and second stages juveniles (j2). These j2s have a stylet of which the cone is straight and usually longer than the shaft (stylet length (9-23µm), the shaft may narrow in front of the knobs, relatively large median bulb, long oesophageal overlap and a tapering tail with distinct irregular cuticle inclusions and finely rounded tip. The differentiating characters for the female are entire body annulated, head region set off, stylet small (10-25µm lining; in majority of species (11-17µm long), excretory pore anterior to metacarpus, perineal pattern present vulva and anus terminal Males are characterized by distinct amphideal openings, short tail, caudal alae (bursa) absent (Moens, 2009).

2.3.3 Symptoms of diseased tissue after root-knot nematode attack

Within 24hrs after larvae enter the root, cortical cells near the larva undergo pronounced hypertrophy, especially if several larvae have entered simultaneously. Cells of the Pericles and endodermis when lying near the path of larvae will show slight hypertrophy. Usually a root may continue to grow after infection, sometimes growth is retarded or even halted and the resulting gall will be located pendulum like at the end of the root. The presence of the nematode apparently stimulates mitotic activity in the Pericles. Once this cell division is initiated it proceeds in a normal manner and lateral roots may be formed. This would explain the high frequency with which lateral roots occur at the point of infection. Mature root-knot females deposit eggs in a gelatinous matrix which may hold as many as 2000 eggs. Within 3 days after penetration by the larvae usually 3 to 6 stellar host cells lying in the region of the nematodes head begin to swell and form giant cells. In general then, root-knot galls are characterized by knots of distortion and broken vessels, surrounded by fleshy tissue, which may be discolored and furrowed. The result in addition to gall formation is a general degradation of the normal functions of the affected plants (Marais and Fourie, 2010)

2.3.4 Physiological effects of root-knot nematode attack on the host

The nematode salivary secretions injected into the host tissue probably include a mixture of enzymes and hormones that directly

1stimulatethe plant to develop certain special cells on which the nematode can feed

2 Stimulates divisions of cells

3 Cause cellular hypertrophy

4 Suppresses cell division in the apical meristematic and roots and shoots

5 Dissolve or digest the middle lamella and the cell walls

6 Stimulate enzyme productions and the accumulation of high levels of protein or the secretion trigger a plant mechanism that initiates these abnormal growth processes. Much of the damage that root-knot nematode inflict upon plants results from the type and extent of the biochemical response of the surrounding tissues to nematode stimulus (Lucas 1975).

2.3.5 Factors affecting Distribution of root knot nematodes

Moisture status, aeration, texture and temperature of soil are major physical factors that affect the distribution and reproduction of root-knot nematodes and the damage they cause to host crops. J_2s migrate through soil water films and are most active at soil moisture levels of 40-60%of field capacity (Wang et al 2002).

2.3.6 Economic importance of the root knot nematodes in tobacco production

Root – knot is a primary disease threat to tobacco production in many countries. Loses from root – knot are heavy, in combination with other pathogens the disease can be disastrous. Root-knot nematode damage was found to be high in sandy loamy soils characteristic of the soils in which tobacco production is practiced in Zimbabwe (Tobacco Annual report, 2005). Research must continue if we are to find new and more efficient ways to control these ever–

present parasites. This genus is regarded as the economically most important plant-parasitic nematode group in the tropical regions (Marais and Fourie, 2010).

2.4 Signs and symptoms of root knot nematode damage

2.4.1 Above ground symptom

The damage caused by nematodes is often non-specific and easily confused with symptoms of other abiotic or biotic stresses, (Coyne et-al; 2007). Symptoms of above-ground parts of the plant may include various degrees of stunting, lack of vigour and wilting under moisture stress (Moens et al, 2009). During periods of drought or excessive water loss affected plants develop severe symptoms. These plants become stunted and show a marked tendency to wilt during the hot part of the day. Plants may appear chlorotic, stunted, necrotic, or wilted especially during periods of moisture stress and high temperature. The plants develop a light yellowish cast in contrast to the normal green of healthy plants (Marais and Fourie, 2010). Many leaves particularly those at the middle and lower parts of the plant, show yellow necrotic margins and tips thus destroying one third one half of the leaf area pronounced flagging of such leaves is followed by premature yellowing and “ringing” of the older leaves which must be harvested green if at all. This makes poor quality product and results in heavy loss to the grower (Kleynhans et al, 1996). Fig 2.1 shows the above ground symptoms of root-knot nematode damage.

Fig 2.1 Showing above ground symptoms of root-knot nematode *(Meloidogynespp)* damage adopted from (Powers and Mcsorley, 2000)

2.4.2Below ground symptoms

Nematodes are important yield reducing pests of most agronomic crops worldwide. Nematodes typically feed on subterranean plant tissues including roots, tubers and rhizomes Walker et al (2002). Diagnostic symptoms appear on roots and also hypocotyls of infected plants in the form of galls in the roots, Galls are 1-10mm in diameter or larger depending on species and location in the rooting system Hall (1991); Moens (2009).The most distinctive symptoms of root–knot are the galls on the roots. The galls vary in size from a pinhead to many times the thickness of the affected root on which they grow. In shape they are irregular, spindle shaped, or spherical. Although root-knot nematodes usually infest underground parts of the host, plant they are occasionally invading stems and leaves of certain plants, for instance African violets (Marais and Fourie, 2010)

The size and shape of galls differ somewhat with different nematodes spp. Although the knots may be scattered on any part of the main root or its branches, they are most often

found on tender rootlets, resembling beads on a string. However the galls are sometimes so close together they appear to be a single elongated gall. Small galls contain at least one nematode while large galls may contain numerous larvae in all stages of development. Adult females in dissected galls appear as minute, pearly white, rounded bodies, from one fourth to one half the dram of a pinhead. Eggs, deposited in a gelatinous mass, accumulate outside the posterior end of the body of the female. Often the cortical tissue of the root is broken, after which the eggs are pressed out and appear as light brown masses which are barely visible at the surface of the root. The galls in particular the giant cells, provide a favored site for the development of soil of soil bourne fungi, often enabling the pathogen to overcome resistance that the plant should have in the absence of succesful root-knot nematode invasion. Fig 2.2 show the characteristic symptoms of root-knot nematode damage.

Fig 2.2 Showing below ground symptoms of root-knot nematode *(Meloidogyne javanica)* damage adopted from (Duniway, 2002)

2.5 Means of spread of root knot nematodes

Root-knot nematodes are now widespread in most of the warmer tobacco growing regions of the world that there is little hope of eradicating them. Man himself has been of principle responsibility for this spread of the root-knot nematode. Transport of root-knot infected transplants has contributed to its introduction and spread. Nematodes are unnoticed contaminants in the soil sticking to plant roots, tools vehicles or the feet of animals. Drainage and irrigation water are also important factors in spread; eggs and larvae may be carried long distances in surface water to begin new caners of infection. In dry weather strong winds may blow the eggs from one field to another. Ordinarily the nematode larvae travel only a few cm through the soil by their own effort (Kleynhans, 1996).

2.6 Management Strategies of root knot nematodes

The root-knot nematode is so widespread in many tobacco producing areas that it is particularly impossible to eliminate this pathogen from infested fields. The aim of management practices implemented must not focus only keeping the nematode population at minimal quantities but to prevent the built-up of any one species to a point where it could do great damage. The management practices include; Chemical, Biological, Varietal resistance and cultural control

2.6.1Chemical control of root knot nematodes

A great discovery in Zimbabwe came with the introduction of Dichloropropene Dichloropropane by Daulton in 1949 just after the 2nd world war by 1954 EDB and methyl bromide where also found to be effective fumigants. These common controls were accompanied by a broad base of other chemicals until their hazardous effects on the environment and humans were established (Desager et al, 2006). Most of these chemicals including methyl bromide and EDB are currently in the process of world wide phase out (Duniway, 2002), with the developing countries being given a grace period of up to 2015 to face them out. To date efforts are being made to introduce more environmentally friendly

chemicals with test on methyl iodide and sodium being tested in seedbeds for efficacy at Kutsaga.

2.6.2Varietal resistance in root knot nematode management

Efforts have been tirelessly made to breed cultivars that are resistant to the root-knot nematode since these nematodes are responsible for 70% of nematode attacks on tobacco. The Tobacco Research board made a major break through when it managed to release flue cured varieties that are resistant to the nematodes. *N. repaunda* and *N. longiflora* were found to be sources of resistance to *Meloidogyne spp* and therefore their hybrids produce higher yield and quality of tobacco (Tobacco annual report 2008).

2.6.3 Biological control of root knot nematodes

Nematode populations can be regulated or reduced by employing organisms antagonistic to the root-knot nematode, this form of control is referred to as the biological control. Biological control indicates the use of control agents in which the active principle is a living organism or virus, they are often referred to as natural enemies for the regulating the incidence of pests and pathogens (Oudejans, 1991). The most used biological control agents are fungi and bacteria, examples of which are *Paecilomyces ulacinus* and *Pasteuria penetrans* respectively . These where investigated and found to be effective. *Pochonia chlamydosporia* a fungi has been intensively investigated as a biological control, it is a wide spread facultative parasite in the soil. Pochonia has been found to be effective though it is still being tested at Kutsaga for suitability to the ecology. Fungi and bacteria are mainly used with examples of the nematode feeding fungi such as *Pochonia spp, Chlamydosporia* and *Paecilomyces* (Oudejans, 1991).

2.6.4 Cultural control of root knot nematodes

The use of cultural method is the most environmental sustainable and potential most successful method for controlling root knot nematodes damage. The methods are crop rotation with non-host crops for example Katambora Rhodes grass, phyto-sanitation in

seedbeds and the use of legislation for example crop planting and destruction dates (Verdejo-lucas*et-al.*, 2003).The suspension of several important nematicides has prompted investigations into alternative, non chemical methods for the management of plant- parasitic nematodes. Cultural control measures aim at modifying the cropping environment to the extent that it becomes less favorable for the development of the pest populations whilst maintaining the best possible conditions for high productivity.

The use of cultural methods in controlling nematodes is one of the oldest strategies implemented from very early years, particularly because they are more economic and environmentally suited than all the other control. Examples of cultural controls include rotations, solarization, the use of cover crops and agronomic practices like early planting, stock destruction and ploughing. Solar heating aims at increasing temperatures to above the nematode optimum survival temperature by covering raised and moist beds with clear plastic during the hottest months of the year (Ploeg, 2001) for a period of at least two weeks. Off the rotation crops tried sunn hemp and Katambora were found to be effective and are still recommended for use. Crop rotation one of the most useful practices that can realistically be applied to annual and short term perennial crops (Biljon, 2010).

2.6.4.1 Crop rotations in the management of root knot nematodes

Crop rotations and resistant cultivars have been and will continue to be a primary means of managing nematode population densities in annual crops (Gains, 2010). Crop rotation is the traditional method of growing in the same field various alternatively over succeeding years with or without leaving the land cultivated (fallow) over intermittent periods of time. The principle of rotating cropping pattern is to interrupt the relationship between pests and host plants which favors or limits the development of these damaging organisms hence an effective measure against soil – borne pathogens, nematodes and soil insects provided the

rotation cycle is sufficiently long (Oudejans, 1991). Crop rotation with non host plants is effective at reducing root galling among susceptible vegetable crops, (Summer et-al, 1999).The ultimate goal being the establishment of a rotation system that allows for the recovery of the soil after a certain crop and with the ability to resist diseases of economic importance to a particular crop for instance root-knot (*Meloidogyne javanica*) in tobacco. A possible crop rotation would be: susceptible > poor host > poor host > non-host or resistant host > susceptible.

2.6.4.2 Tobacco - Sunn hemp rotation

Sunn hemp, *Crotalaria juncea* L. is a rapidly growing crop that is used for fiber production and it is most popular as a green manure in many tropical and subtropical areas in the world as an organic nitrogen source (Marshal, 2002). Sunn hemp suppresses weeds, slows soil erosion, and reduces root-knot nematode populations. Most of the plant-parasitic nematodes suppressed by *Crotalaria* are sedentary endoparasitic nematodes, which are nematodes that remain and feed in one place within the root system (Wang *et al*., 2002). Sunn hemp uses different modes of action to suppress plant parasitic nematodes, making it an efficient cover crop for nematode management. Sunn hemp is not only a poor host or non-host to plant-parasitic nematodes, but it has been shown to produce allelopathic (toxic) compounds against nematode pests. Sunn hemp also can enhance natural enemies of plant-parasitic nematodes, such as fungi (Wang and Mcsorley, 2011) and also suppress plant-parasitic nematodes indirectly, by increasing plant tolerance against these pests. Sunn hemp also enhances free-living nematodes in the soil that are involved in nutrient cycling (Wang et al, 2003), thus increasing nutrients available for plant uptake.

2.6.4.3 Tobacco-Winter Katambora Rhodes grass (*Chloris gayana* Kunth) rotation

The most extensively sown pasture grass is Katambora Rhodes grass (*Chloris gayana* Kunth). Katambora is grown in rotation with tobacco to control Meloidogyne javanica (root-knot nematode). Potential area of use is in the region of 200,000 to 250,000 ha with annual reseeding of up to 60,000 ha. Katambora, a diploid Rhodes grass, came into use in the nineteen fifties in Zimbabwe. It is less productive and palatable than tetraploid strains such as Zimbabwe Giant. Low night temperatures have been associated with poor seed set in Rhodes grass. In Zimbabwe low night temperatures (below 10º C) occur in many parts of the tobacco - hence Rhodes grass - areas by mid-April. A four year ley is recommended to overcome the longevity of nematode eggs even in bare fallow soil (Nyamadzawo and York, 1999). However breeding efforts are currently under way to develop katambora Rhodes grass that requires a three month ley period to break the longevity of nematode eggs (Guerena, 2006)

2.6.4.4 Tobacco - Sugar bean (*Phaseolus vulgaris*) rotation

Crop rotation is of great importance in the production of flue-cured tobacco. Rotation crops, such as beans and tomatoes, encourage the early establishment of destructive soil-borne diseases (Gaines et al 2010). However most farmers would prefer to grow rotation crops that they can either consume or sell, as a result the susceptibility of the root-knot nematodes needs to be evaluated. In addition sugar beans have achieved a well deserved reputation particularly amongst small scale farmers as a poor mans mean (source of protein), it is essential to establish the extent of susceptibility of the root-knot nematode in tobacco rotations. Root knot nematode galls can be confused with *Rhizobium* (Nitrogen fixing bacteria)nodules on roots of leguminous plants. On close examination, however, the nematode gall can be seen to occupy the entire circumference of the root, whereas the nodule is located on the side of a root. (Report on plant diseases, 1993)

2.7 Integrated pest management of root knot nematodes

No single method can effectively manage nematode and as such a combination of controls must be used if crops are to be grown profitably. IPM is a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and it maintains the pest populations at levels below those causing economic injury. (Rivard et-al, 2010) argued that the integrated pest management approach seeks to reduce damage caused by soil borne pathogens without the use of chemical fumigants however (Oudejans, 1991) suggested that it is an economically and sustainable system of crop protection consisting of a combination of cultural, biological, genetic and chemical control methods, that aim at maximizing productivity with the least possible adverse consequences to the environmental.

2.8 Spatial and temporal distribution of root knot nematodes

Nematodes migrate up and down the soil profile in response to moisture and host availability (Ferris, 1966), this complicates management decisions as the nematodes found at deeper profiles might escape treatment and later migrate up the soil profile causing damage to the tobacco crop. It is essential to sample at the appropriate sampling depth and time for accurate nematode populations to be established in an area and for the appropriate management strategy to be implemented.

This study will go a long way towards toward attaining information on the behavior of root-knot nematodes in space and time, under different rotations and fumigation types. The nature of this study is that it is fundamental and as such it will unveil new possibilities in nematology as well as pave way for a more integrate pest management approach (IPM).

CHAPTER THREE

3.0 Materials and methods

3.1 Study site

The study was carried out at Kutsaga Research station which is located 15km east of Harare. It is located 31'08"East and 17'55" South. The station falls in the natural region IIB and the annual rainfall is between 800-1000mm. The temperature range is 18-32ºC. The soil type consists of granitic sandy loamy soils with the main clay component being kaolinite (Nyamapfeni, 1991).

3.2 Experimental design and treatments

The experimental design was a split plot in a randomized complete block design, with three replications. Fumigation was the main plot factor and rotations as the main plot factor. The rotations were (sugar beans – tobacco), (Sunn hemp –tobacco), (Winter Katambora – tobacco) and (winter fallow – tobacco). The blocking factor was slope.

3.3 Varieties

The tobacco cultivar used was KRK26, which was released in 2001 and is a common cultivar amongst tobacco farmers' especially small scale. KRK26 is a medium ripening cultivar which does well in all areas and it has a medium to high yield potential. The Katambora grown was Katambora Rhodes grass HRG1. This is a new Katambora Rhodes grass (*Chloris gayana* Kunth) variety with nematode tolerance properties.

3.4 Agronomic practices

The raising of tobacco seedlings was done using the float tray system of seedling production. The fertilizer Floatfert (Hydrofert) was applied at 7, 21 and 35 days after sowing respectively.

Before transplanting the seedlings were hardened through a reduction in water and nutrient application to prepare them for field conditions. Transplanting was done when seedling stems reached a height of 15 - 17cm and a thickness of 6 - 10cm. Water planting was done when the seedlings were transplanted into the field. Each row had 32 planting stations. Chlorpyrifos ® application for cutworm control was done at planting.

Deep ploughing was done using a tractor-drawn plough to a depth of 38 cm and ridges were constructed. The tobacco variety KRK26 was grown on ridges because ridges promote good surface drainage and provide a good environment for early growth of the crop. Fumigation was done using Ethyl dibromide EDB 98%, at a rate of 125ml per 100m ridge in the fumigated treatments. Plants were planted at a spacing of 120cm × 56cm apart. Weed control was done manually since some herbicides have nematode control properties. Agronomic practices including gap filling, topping, suckering and reaping were done at recommended times.

3.4.1 Nematode sampling period

The sampling period for this experiment was from September 2010 to April 2011. Sampling was done once every month.

3.5 Sampling procedure and data collection

Sampling was done once at the beginning of every month from September 2010 to April 2011. Sampling was done in three blocks. The total number of treatments in each block was eight. Therefore the number of plots in each block was also eight. In each treatment sampling was done in the middle row to avoid edge effects. Three positions were sampled in the middle row of each treatment to obtain a good representation of the plot (see diagram 1). At each sampling position 3 samples were collected at intervals of (0-15cm), (15-30cm) and 30-45cm) respectively. A sampling auger was marked at 15cm intervals for the 3depths or profiles. During the sampling process at one sampling position, the 1st (0-15cm) was sampled

and the sample transferred into a bucket then into a polythene plastic sampling bag. The samples were marked with sampling pegs to represent the block, plot and treatment number, to avoid mixing of samples. After the 1st sample was collected and the (0-15cm) mark on the sampling auger was reached, at the second sampling position, the 2nd (0-15cm) was collected and at the third sampling position, the 3rd (0-15cm) sample was collected. After all positions had been sampled in the 0-15cm depth the sample was mixed and transferred into a sampling bag with a marked peg to represent the treatment number and block. The other two depths were sampled in the same way as the 0-15cm depth. This sampling procedure was done for the 8 treatments in each block. After ridging, the ridge constituted a small part of the top 15cm due to the slight elevation above the ground. To insure continuity of the experiment the ridged part was referred to as depth z, the ridges were only found in the plots planted to tobacco during the course of the tobacco growing season. At the end of the tobacco growing season the ridge (profile z) was destroyed. This was done to insure continuity in the measurements made for the three depths in all plots/treatments throughout the course of the experiment.

Sugar beans, Sun hemp and the winter Katambora were planted in March after tobacco (KRK26) had been harvested from the 2010-2011 tobacco growing season. The sampling procedure is as illustrated in Fig1.

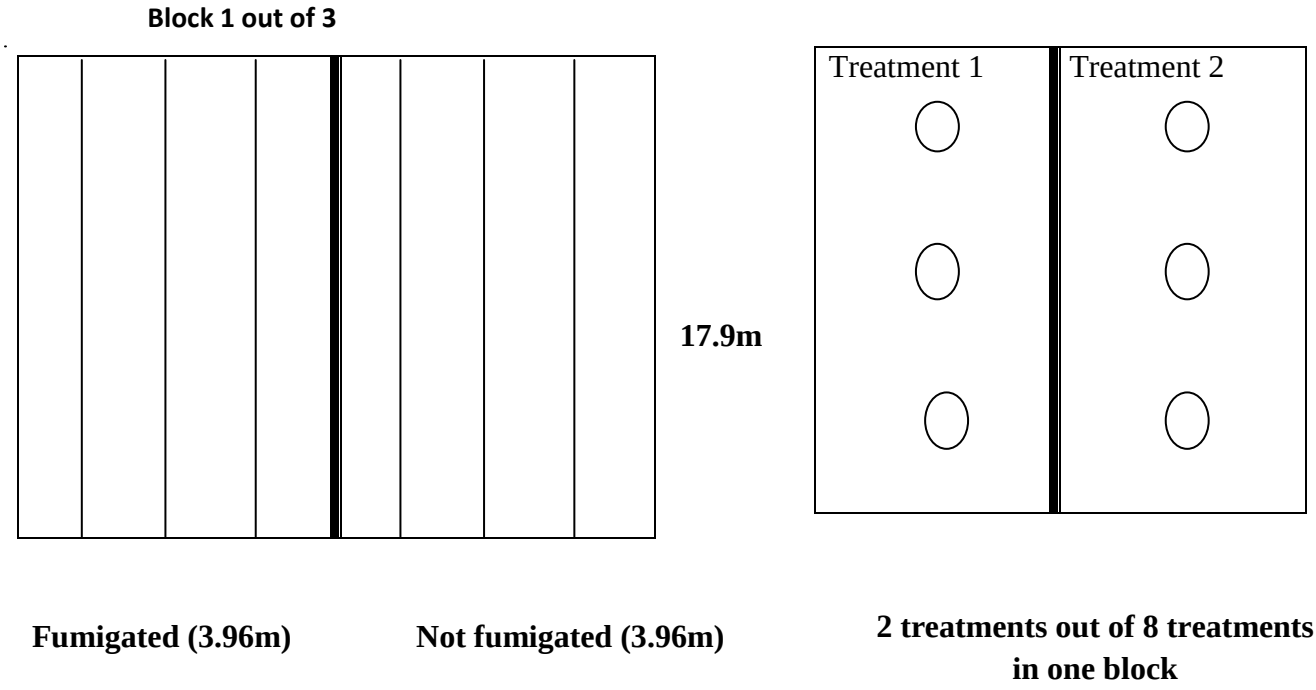

Fig 3.3 Diagrammatic illustration of the sampling frame and sampling procedure in each treatment/ block

3.6 Analysis of samples

3.6.1 Bioassays

After sampling was completed, the samples were taken to the green house for the set up of bio assays. Bioassays are an indirect method of measuring the population of nematodes at a specific time. Cultures were set up using tomato plants (*Lycopecicum esculenta)* due to their high susceptibility to nematodes. Tomatoes act as indicator plants for root-knot nematode damage. The samples were transferred into pots and tomato seedlings raised in fumigated soils were transplanted into the sampled soil. Each pot was marked with a peg to show the block and treatment the sample represented and placed in each pot to avoid mixing up of samples. The principle behind the culturing of tomatoes in sampled soil was that the nematodes in the sampled soils would attack the tomato seedlings since the tomatoes are highly susceptible to nematode attack. The tomato plants were irrigated twice a day. Spraying of the tomato plants was done every week to prevent the spread of red spider and white fly. Bioassays were assessed after 5-6 weeks, 5 weeks in summer and 6 weeks in winter and the

damage was rated using Daulton's gall rating scale (1961) which ranks nematode damage on a scale of 0-8 (Appendix 1)

3.7 Statistical Analysis

The data was transformed using log transformation before it was entered into the statistical package Genstat version 7.22 for Analysis of variance (ANOVA) at 5% level of significance. The least significant difference (LSD) was used to separate treatment means where there was significant difference.

CHAPTER 4

4.0 Results

4.1 Nematode distribution at different soil depths for the rotations (Sugar beans, Sunn hemp, Winter Katambora and winter fallow)

No interactions were recorded between rotations and fumigation ($p>0.05$), However, significant differences were recorded for the different tobacco rotations. Sunn hemp recorded the lowest nematode populations in all three depths (0-15cm), (15-30cm) and (30-45cm) throughout the experiment.

Fumigation showed no significant differences in nematode activity throughout the experiment in the three depths. (Appendix 2, 3, 4)

4.1.1 Nematode distribution at 0-15cm depth

The results in table 4.1 show nematode distribution for the 0-15cm profile. Significant differences in nematode activity for the different rotations were recorded in September. Table 2 shows significant differences in nematode activity with the sugar bean rotation being significantly different from the sunn hemp, winter fallow and winter katambora rotations. The other crops did not vary significantly from each other in nematode activity in the (0-15cm depth). The results were significant at both 1% and 5% level of significance. Appendix 2 shows results for the ANOVA performed for the 0-15cm profile.

Table 4.1: Bioassays for depth 0-15 cm

CROP	MEAN BIOASSAYS SEPTEMBER
Sugar beans	**0.48a**
Sunn hemp	**0.05b**
Winter fallow	**0.05b**
Winter Katambora	**0.13b**
Grand mean	**0.79**
SE	**0.09**
CV %	**69.7**
LSD **F probability**	**0.89** **0.001****

4.1.2 Nematode distribution at 15-30cm depth

The results in table 4.2 show nematode distribution for the 15-30cm profile. Significant differences for the different rotations were recorded in March. The winter Katambora rotation was significantly different form the beans and winter fallow and sunn hemp rotation, however the latter rotations were not significantly different from each other. Appendix 3 shows results for the ANOVA performed for the 15-30cm profile.

Table 4.2: Bioassays for depth 15-30 cm

CROP	MEAN BIASSAYS MARCH
Sugar beans	**0.19a**
Sunn hemp	**0.15a**
Winter fallow	**0.23a**
Winter Katambora	**0.38b**
Grand mean	**0.24**
SE	**0.05**
CV %	**39.4**
LSD **F probability**	**0.12** **0.007****

4.1.3 Nematode distribution at 30-45cm depth

Nematode distribution in the depth (30-45cm) is shown in table 4.3. Significant differences between rotations were recorded in January. The winter katambora rotation is significantly different at 5%. The results showed significantly high nematode activity in the winter Katambora rotation compared to the sunn hemp, winter fallow and sugar bean rotation at 30-45cm depth, however the latter rotations were not significantly different from each other. Appendix 4 shows results of the ANOVA performed for the 30-45cm profile.

Table 4.3: Bioassays for depth 30-45cm

CROP	MEAN BIOASSAYS JANUARY
Sugar beans	**0.00a**
Sunn hemp	**0.05a**
Winter fallow	**0.08a**
Winter Katambora	**0.23b**
Grand mean	**0.09**
SE	**0.06**
CV	**67.2**
LSD **F probability**	**0.13** **0.02***

4.2 Temporal distribution of root-knot nematodes in depths 0-15cm, 15-30cm and 30-45cm.

Fig 4.4 shows the results for the temporal distribution of nematodes in the three depths, it showed that nematode populations fluctuated throughout the course of the experiment. Between the months of September and November, high nematode activity was recorded in the 15-30cm and 30-45cm profile. Nematode populations were low in the 0-15cm profile.

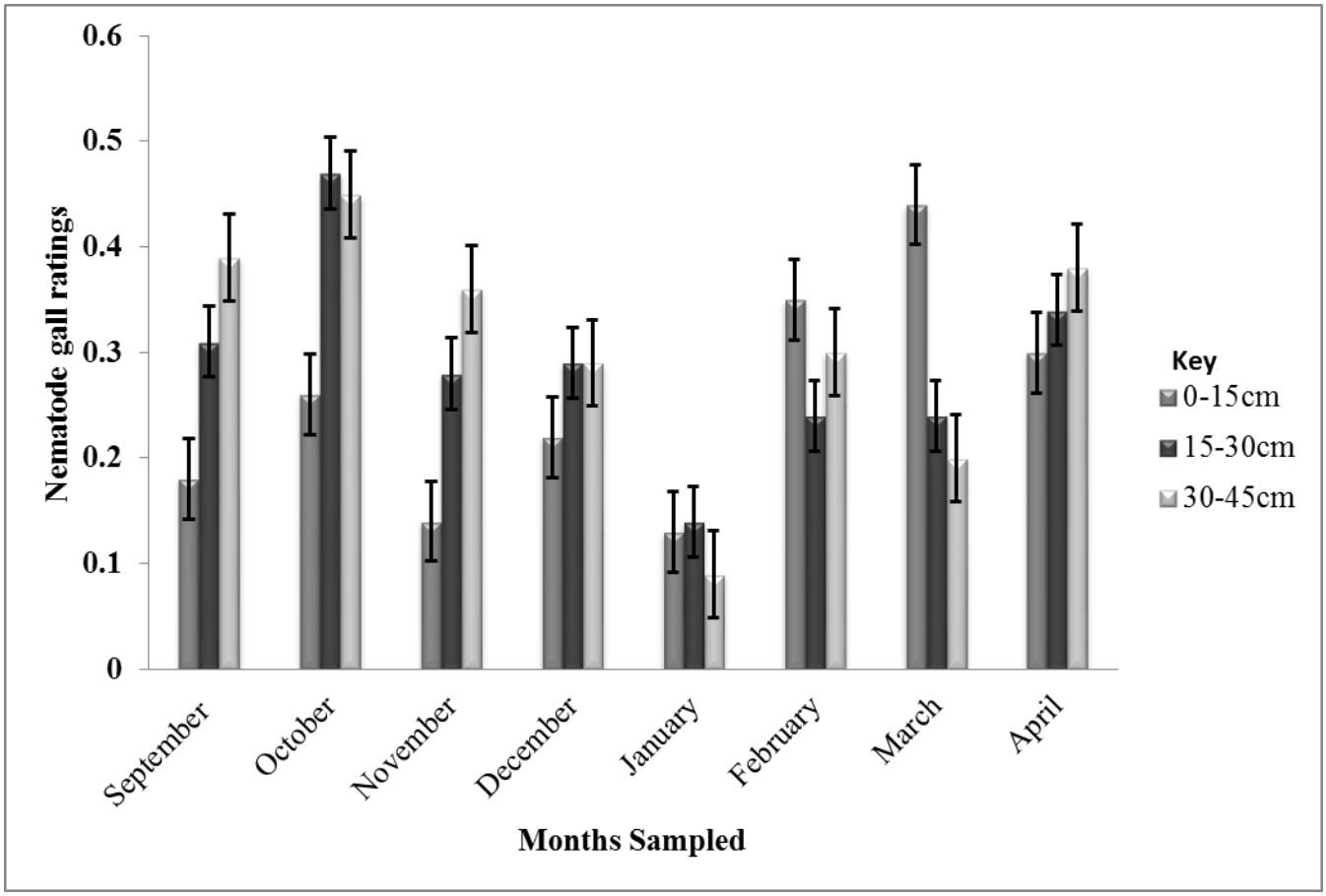

Fig 4.4 Temporal distribution of nematodes from September to April in the 0-15cm, 15-30cm and 30-45cm profile

CHAPTER FIVE

5.0 Discussion

5.1 Effect of rotation type and fumigation on the spatial and temporal distribution of nematodes.

The results showed nematodes migrated up and down the soil profile throughout the course of the experiment. This complicates management decisions as nematodes found at lower depths of the soil profile might escape treatment and migrate up the soil profile during the course of the tobacco growing season.

The populations of nematodes found in this experiment might be attributed to the fact that prior to the establishment of this experiment; the field used was planted to a fumigated tobacco crop (variety T66). This variety has high nematode resistance and as a result it might have lowered nematode populations, (Tobacco hand book, 2011).

In addition the tobacco variety KRK26 which was planted in the experiment. This cultivar is a common cultivar amongst tobacco farmers particularly the small scale farmers due to its early ripening or maturity and easy management. However it has some level of tolerance to root-knot nematodes.

5.2 Nematode distribution in the rotations winter fallow, winter Katambora, sugar beans and Sunn hemp in the three depths.

5.2.1. Nematode distribution in the 0-15cm profile

The results for the 0-15cm profile showed significant differences in nematode distribution for the different rotations. The (sugar bean - tobacco rotation) had significantly higher nematode counts than Sunn hemp, winter fallow and winter Katambora rotations. This could have resulted from the fact that the sugar bean rotation is more susceptible to the root-knot nematodes than any of the other rotations. This is supported by a previous study carried out by Summer in (2003) in a study to evaluate the effect of root diseases and root-knot

nematodes on vegetable rotations and came to the conclusion that the sugar bean was susceptible to nematodes. In another study carried out by Gaines et al (2010), it was reported that rotation crops, such as beans and tomatoes, encourage the early establishment of destructive soil-borne diseases due to their susceptibility.

5.2.2 Nematode distribution in the 15-30cm profile

The results for the 15-30cm profile showed significant differences in nematode activity for the different rotations. The populations were highest in profile in October. The winter (Katambora-tobacco rotation) had significant differences from the other rotations with a mean of 1.83. These results suggest that the winter Katambora might have short lived nematode control properties since the current standard Katambora tobacco rotation consists of a three year period were by the Katambora would be planted in the tobacco field before tobacco is planted. These results support the findings of York and Nyamadzawo, (1999). They concluded that a 3-4 year ley period was recommended to overcome the longevity of nematode eggs even in bare fallow soil. As a result the winter katambora which is planted for three months (during winter) before the next tobacco growing season might not be able to break the longevity of nematode eggs.

5.2.3 Nematode distribution in the 30-45cm profile

The results for the (30-45cm) depth showed significant differences in nematode distribution for the different tobacco rotations. In October as is the case for the (15-30cm) Depth nematode populations were also highest. The winter katambora rotation showed significantly higher nematode activity than the other rotations for the same reasons mention above in the 15-30cm profile.

5.2.4 Nematode distribution in the tobacco- sunn hemp rotation in the three depths

The tobacco Sunn hemp rotation recorded the lowest nematode populations in all the three depths compared to the other rotations. This might be attributed to the fact that in previous studies, Sunn hemp was found to suppress weeds, slows soil erosion, and reduces root-knot nematode populations (Marshal, 2002). These findings were also supported by (Wang et al, 2003) when he stated that sunn hemp is not only a poor or non-host but it uses different modes of action to suppress plant parasitic nematodes, which include the release of allelopathic chemicals. These properties make it an efficient cover crop for nematode management.

5.3 The temporal distribution of nematodes in the three soil depths

The results for the temporal distribution of nematodes showed high nematode activity between September and November in the 15-30 and 30-45cm profiles. At this time nematode populations were low in the 0-15cm profile. Sampling at this time would give an indication that nematode populations in an area are low because the recommended sampling depth is between 0-15cm. High nematode populations were located in the 0-15cm profile between march and April. Sampling at this time would give a true indication of the nematode population in an area. This is the current recommended time to sample as stated by the (Tobacco hand book, 2011)

CHAPTER SIX

6.0 Conclusion and recommendation

6.1 Conclusion

There were significant differences recorded in the distribution of nematodes for the different tobacco rotations. Fumigation did not affect nematode distribution in the (0-15cm), (15-30cm) and (30-45cm) soil profile. The nematode populations differed at the three depths from September to April, with the lowest nematode populations being recorded in January and the highest populations in March. Winter Katambora showed a build-up in nematodes in depths (15-30cm) and (30-45cm) compared to the other crops. Sugar beans recorded significantly higher nematode activity than the other rotation crops in the (0-15cm) soil profile.

6.2 Recommendations

Based on the results of this study it is recommended that soil sampling for nematode counts be done in March since nematode populations have been found to be highest in the (0-15cm) depth during this month and since sampling is usually done in the (0-15cm) depth. Sampling at this time would give the correct indication of nematode activity in the soil. In addition, sugar bean is not recommended for use in tobacco rotations due to its susceptibility to nematodes. It is recommended that the experiment be repeated with the same rotation crops to evaluate the spatial and temporal distribution of nematodes in tobacco rotations. The experiment should also be carried out using other tobacco cultivars instead of just one cultivar KRK26. The sampling depth could also be increased to achieve greater variations in nematode distribution.

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LIST OF APPENDICES

APPENDIX 1. Root Knot Nematode Infection Indices (Daulton's gall rating scale)

Infection class	Index value	Description of degree of galling on roots of indicator plants
0	0	Free from galls
1	1	Trace infection, less than 5 galls
2	5	Very slight, trace to 25 galls
3	10	Slight, 26 to 100 galls
4	25	Moderate, numerous galls, mostly discreet
5	50	Moderately heavy, numerous galls, many coalesced
6	75	Heavy, very numerous galls, mostly coalesced, root growth slightly retarded
7	90	Very heavy, mass invasion, slight root growth
8	100	Extremely heavy, mass invasion, no root development

APPENNDIX 2: Analysis of Variance for September Bioassays: Depth (0-15cm).

SOURCE OF VARIATION	**D.F.**	**S.S.**	**M.S.**	**V.R.**	**F prob**
Block stratum	2	4.333	2.167	1.86	
Block. Fumigation stratum					
Fumigation	1	0.042	0.042	0.04	0.868
Residual	2	2.333	1.167	0.77	
Block. Fumigation. Rotation stratum					
Rotation	3	19.4583	6.4861	12.49	<.001
Residual	15	7.7917	0.5194		

Total	23	33.9583

APPENDIX 3: Analysis of Variance for March Bioassays: Depth (15-30cm).

SOURCE OF VARIATION	**D.F.**	**S.S.**	**M.S.**	**V.R.**	**F pr.**
Block stratum	2	15.0833	7.5417	0.59	
Block. Fumigation stratum					
Fumigation	1	20.1667	20.1667	2.23	0.274
Residual	2	18.0833	9.0417	20.34	
Block. Fumigation. Rotation stratum					
Rotation	3	4.3333	1.4444	5.91	0.007
Residual	15	3.6667	0.2444		
Total	23	61.3333			

APPENDIX 3: Analysis of Variance for January Bio assays: Depth (30-45)

SOURCE OF VARIATION	**D.F.**	**S.S.**	**M.S.**	**V.R.**	**F prob**
Block stratum	2	2.0833	1.0417	1.39	
Block. Fumigation stratum					
Fumigation	1	1.5000	1.5000	4.00	0.184
Residual	2	0.7500	0.3750	1.35	
Block. Fumigation. Rotation stratum					
Rotation	3	2.3333	0.7778	4.37	0.021
Residual	15	2.6667	0.1778		
Total	23	9.3333			