EVALUATION OF ORGANIC AMENDMENTS FOR THE MANAGEMENT OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

KNUST

BY

FAYE JERREH MANNEH

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

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A Thesis submitted to the DEPARTMENT OF CROP AND SOIL SCIENCES.

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DOCTOR OF PHILOSOPHY

IN

NEMATOLOGY

BY

FAYE JERREH MANNEH (MPHIL NEMATOLOGY)

NOVEMBER, 2016

DECLARATION

I, hereby, declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Root-knot nematodes are considered as one of the most economically damaging of plant-parasitic nematodes of tomato. In many cases, crop losses due root-knot nematodes are reduced by the application of high toxic chemical nematicides. In Ghana, farmers‟ management practices against root-knot nematodes are in most cases ineffective. The purposes of the study were to determine farmers‟ perception of rootknot nematodes, evaluate the efficacy of organic amendments at the laboratory, plant house and field,assess the effect of fresh sweet orange and cassava combination on root-knot nematode isolates, confirm the presence of the nematodes (*Meloidogyne* spp.) using ITS-PCR molecular analysis,determine the time of application and appropriate rate for the management of the nematodes on tomato. In determining farmers‟ perception of root-knot nematodes and their management methods, multistage sampling technique was used to select the respondents. The survey was carried out in six district in Ashanti region. Majority (30%) of the tomato farmers were aware of root-knot nematode problems in their fields. Majority of them identified root-knot nematodes as the most prevalent pest in tomato. However, none of them used nematicides in the management of the nematodes. All the farmers used crop rotation and weeding as a management strategy against the nematodes. For the evaluation of the efficacy of organic amendments in the management of the nematodes, experiments were carried out in the laboratory, plant house and research field of KNUST. In the laboratory, fresh sweet orange peels significantly ($P<0.05$) inhibited egg hatching and increased mortality of root-knot nematodes than the rest of the treatments. Fresh orange peel performed just as well as carbofuran in reducing the number of root-knot nematodes in the root of tomato and soil, number of eggs, root galling and reproduction factor of the nematodes in the plant house. In the field, the application of fresh sweet orange peels followed by cassava peels and the reverse application of the two materials significantly increased ($P<0.05$) the fresh shoot weight, fruit size and yield of tomato than the other treatments. The number of rootknot nematodes in the roots of tomato and soil, number of eggs and root galling were significantly reduced ($P<0.0$) upon the application of carbofuran and its effect was similar to that of the combined application of fresh sweet orange and cassava peels. The application of sweet orange peels significantly increased ($P<0.05$) the number of colonies of *Trichoderma viride* than cassava peels, but cassava peels also significantly increased ($P<0.05$) the number of the bacterivorous

nematodes,*Heterocephalobellus* sp. and*Eucephalobus* sp. and the fungivorous nematode*Ditylenchus*sp.more than sweet orange peels. Further evaluation of the combined application of fresh sweet orange and cassava peels on root-knot nematode isolates showed no significant differences (P>0.05) between the isolates in terms of number of juveniles in the roots of tomato and soil, number of eggs, root galling and reproduction factor. However, the pots treated with combined application of fresh sweet orange and cassava peels significantly increased (P<0.05) the fresh shoot weight and plant height of tomato more than carbofuran-treated pots. The amplification of the ITS regions of the nematodes, using multigene loci primers, confirmed the presence of *Meloidogyne* spp. The application of fresh sweet orange and cassava peels combination at four weeks before transplanting significantly reduced (P<0.05) the population of root-knot nematodes and root galling of tomato than the rest of the treatments. Similarly, the application of 25g each of both fresh sweet orange and cassava peels per pot significantly reduced (P<0.05) the nematode population and root galling and inceased fresh shoot weight and yield of tomato than the other treatments. The study showed that root-knot nematode infestation was one of the major problems of tomato farmers and there was no effective management technique. The combined application of fresh sweet orange and cassava peels at 50g/plant four weeks before transplanting was found to be the most effective treatment in the management of the nematodes. The nematode isolates collected from different tomato growings areas were confirmed to be *Meloidogyne* spp. Therefore, further evaluation on the efficacy, time and rate of application of fresh sweet orange and cassava peels combination is recommended. Also, simple and more reliable method such as isozymes should be used to identify root-knot nematodes to the species level.

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ABBREVIATIONS

ANOVA Analysis of Variance

bp base pair cm centimeter

CV Coefficient of Variation

^{o}C degree Celsius

DNA Deoxyribonuleic acid dATP

Deoxyadenosine triphosphate EBT

Ethylene diamine tetra acid

EDTA Ethylene diamine tetra acetic acid FAO

Food and Agriculture Organisation

h hour

ITS Internal Transcribe Spacer LSD Least Significant Difference

min minute ml millilitre mm millimeter

PCR Polymerase Chain Reaction

rDNA ribosomal DNA

Uv Ultravoilet

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DEDICATION

This thesis is dedicated my dearest wife and children.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

The vegetables sector in most West African countries are recovering from the shocks of policy reforms which are targeting to change the economic strategy to marketoriented economy. These crop products are increasingly becoming important as produce for both local and export markets. They have a great potential to enhance the nutrition status and, thereby, health of consumers as most are good sources of vitamins, minerals and proteins needed for the proper functioning and development of the body (James *etal*., 2010). In Ghana, the government is encouraging the development of the vegetable sector in order to diversify the country‟s export base (GIPC, 2013).

Among the vegetables grown, tomato is one of the most popular with high per capita consumption, used in almost every Ghanaian household (Asare-Bediako *et al.*, 2007). However, crop yields per hectare are 35–50% below potential yields of 20 Mt/ha.The low yield was mainly attributed to the susceptible nature of the crop to insect pests and diseases among which plant-parasitic nematodes are one of the most prevalent (Anang *etal*., 2013).

Plant-parasitic nematodes have been implicated as a major constraint to tomato production (Luc *et al.,* 2005a). Many different genera and species of nematodes are important to tomato production in Ghana. In many cases, a mixed community of plant-parasitic nematodes is present in farmers‟ fields, rather than a single species situation. In general, the most widespread nematode species are the root-knot nematodes(*Meloidogyne* spp.). The root-knot nematodes are economically important group of plant-parasitic nematodes (Perry *et al*., 2009). The common root-knot nematodes identified as parasites of tomatoes in the tropics are: *M. incognita*(Kofoid

and White),*M. javanica* (Treub), and *M. arenaria* (Neal)of which *M. incognita*is the most important (Bridge *et al*., 2005).Previous studies on diversity of root-knot nematodes indentified*M.incognita, M. javanica*and *M. arenaria*as the root-knot nematode species on tomato in Ghana (Kwara *etal*., 2014). Yield loss of about 20.6% in tomatoes has been attributed to*Meloidogyne* species (Luc *et al.*, 2005b).The smallscale farmers on the other hand, mainly use crop rotation and weeding to control nematodes on vegetables. The commercial farmers use broad spectrum nematicides such as carbofuran and Methyl bromide to control root-knot nematodes. However, with the banning of these chemicals, alternative nematode management strategies are required.

The cropping systems used in small-scale farm conditions and in extensive field production, where the use of nematicides is either not economically viable or nonexistent, effective alternatives need to be rationally chosen for management programmes based on economics and reliability.

Organic amendments have been identified to enhance soil fertility and soil microbial populations(Chang*etal*.,2007),aswellas suppress plant-parasiticnematodes (Bailey and Lazarovits, 2003). Therefore, it was against this backdrop that the efficacy of the different organic soil amendments were tested for their potentials in root-knot nematode management.

The main objective of this study was to identify organic amendments with potentials to increase yield of tomato, enhance microbial activity and reduceroot-knot nematode population below the economic thresholds.

The specific objectives were to:

i. assess farmers‟ perception on root-knot nematodes and their management; ii. confirm the presence of *Meloidogyne* spp. in the experiments using ITS-

RLFPs techniques; iii. determine the efficacy of sweet orange peels and cassava peels as amendment in the management of root-knot nematodes *in vitro* and *in vivo*; iv. assess the effect of sweet orange and cassava peels on the chemical properties of the soil as well as the mycoflora and free-living nematodes in the soil;

v. determine the appropriate rate of application of fresh sweet orange and cassava peels in the management of root-knot nematodes; vi. determine the appropriate time of application of the fresh sweet orange and cassava peels in the management of root-knot nematodes.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EconomicImportance of Tomato

Currently, about 40% of the domestic market sales of products in Ghana comes from horticultural cropsin which tomato is one of the most important crops grown in the country (Donkoh *etal.*, 2013). Tomato is cultivated continuously throughout the year.In Ghana, in 2008, the total area cropped to tomato was 16,130ha, and the estimated production was 284, 000 metric tons. In 2010, production level rose by 35,000 metric tons, valuing $129,347,000 (FAO, 2010).

This sector, as a component of agriculture, employs large numbers of people. Other factors that make tomato important so much in this country is that it is a constituent in almost all diets. The fruit is eaten either raw or cooked in the form of soup, jam, ketch-up and salad, among others (Asare-Bediako *et al.,* 2007). It is a good source of vitamins and minerals which help to make a balanced diet for children and pregnant women (Preedy and Waltson, 2008). The leaf is used for medicinal purposes for example to cure ring-worm; a fungal disease that affects the skin (Preedy and Waltson, 2008).

2.2 Constraints in Tomato Production

Tomato production is adversely affected by several factors, which include limited and high cost of labour, climatic factors, inadequate marketing and storage systems and pests and diseases (Srinivasan, 2010).It has been estimated that an average of over 40% of the yield of tomato is lost annually due to diseases and pests (Srinivasan, 2010). Disease pathogens do not only reduce the yield of tomato in terms of quantity, but also reduce quality (Abubakar *et al*., 2004).Tomato is prone to infection by many diseases and pests right from the seedling stage to harvest. Several fungi, insects, viruses,

bacteria and nematodes frequently attack tomato plants as well as fruits (Youdeowei, 2002).

Insects are one of the major constraints in tomato production in the tropics. Sometimes heavy infestation of spider mites (*Tetranychus* spp.), fruit borers (*Helicoverpa armigera*(Hübner)and *Spodoptera littoralis*(Boisd.)) are encountered during the growing season of tomato(Youdeowei, 2002). An average yield loss of about 50% has been attributed to red spider mites on tomato (Elisha *etal*., 2014). The most dangerous insect pests of tomato are those that transmit viral diseases especiallyaphids (M*yzuspersicae*(Sulzer)) for leaf roll, whitefly (*Bemisiatabaci*(Gennadius)) for leaf curl, and thrips (*Thripstabaci*) for spotted wilt virus (Elisha *etal*., 2014).

Nematodes cause serious problems in all tomato growing areas.Majority of the tomato varieties, both cultivated and wild, are parasitized by one or several nematode species (Singh and Khurma, 2007).In Ghana, Osei *etal*. (2011b) observed higher populations of plant-parasitic nematodes on tomatoesthan on *Mucunapruriens* L. and *Tithoniadiversifolia* Hemsl. et Gray. Several species of nematodes attack the crop, but the most predominant species is the root-knot nematode (*Meloidogyne* sp.) (Singh and Khurma, 2007; Kwara *etal*., 2014).

2.3 Economic Importance of Root-knot Nematodes

Root-knot nematodes are obligate parasites which cause significant damage on roots of many plant species (Jones, 2006). Damage caused by root-knot nematodes is related to their population densities in soils at sowing and their reproduction potential (Wesemael and Moens, 2008). Perry *etal.* (2009) stated that "damage thresholds have been established for several crops, where the average is approximately 0.5-2

juveniles/ml of soil (or from the lower limits of detection, over 1000 individuals/500cm^3 of soil)". Sikora and Fernandez (2005) reported that root-knot nematodes are particularly damaging to vegetables in tropical and subtropical countries of the world and cause losses up to 80% in heavily infested fields.

Many crops grown as vegetables mainly tomato, okra and eggplant are susceptible to root-knot nematodes. Estimates of vegetable crop losses due to root-knot nematodes, mainly *M. incognita* and *M. javanica*, have ranged from 17 to 20% for eggplant and 24 to 38% for tomato (Wesemael *etal*., 2011). According to Castillo and Vovlas (2007), in India damage caused by root-knot nematodes on eggplant, tomato and okra were estimated at 18, 54 and 91%, respectively.

In addition, through feeding in the root, root-knot nematodes injure therootwhich allows othersoil-borneplantpathogenstoenter the root(Back *etal*., 2002).Castillo and Vovlas (2007) stated that"the bacterial pathogen,*Ralstoniasolanacearum*(Smith) commonly coexists with root-knot nematodes on tomato". Also, Wesemael *etal*. (2011) indicated that *Fusarium* spp. were found to be in synergy with root-knot nematodes to cause disease on tomato.

2.4 Means of Survival of Root-knot Nematodes*(Meloidogyne*spp.)

Root-knot nematodes are obligate parasites.Therefore, the absence of suitable host plants for long period ultimately leads to their death (Karssen and Moens, 2006). Survival strategies of root-knot nematodes are dependent on abiotic and biotic conditions. In the absence of susceptible crops, however, they often survive on alternative hosts such as weeds (Dufour *etal*., 2003). In general, conditions favourable for plant growth are also favourable for *Meloidogyne* reproduction (Karssen and Moens, 2006). Dufour *etal*. (2003) found that the optimum moisture levels for hatching of *M.*

incognita juveniles from the egg were slightly above field capacity. Survival is mainly influenced by moisture content of the soil and, to a lesser extent, by temperature (Karssen and Moens, 2006). Under adverse condition, where host plants are absent, juveniles will use their energy reserves in the soil as a means of survival(Evans and Perry, 2009). Juveniles and eggs survive period of moisture stress in the state of anhydrobiosis which enables long-term survival in stressful environments (Evans and Perry, 2009). In many genera, the eggs are the survival stage, being protected in a gelatinous matrix (Luc *etal.*, 2005a). Egg masses collected from dry soil contain empty eggs and anhydrobiotic eggs with second stage juveniles in diapause (Karssen and Moens, 2006)).Many nematodes can undergo temporary quiescence in response to environmental stress (Evans and Perry, 2009). The incidence of diapause differs greatly between species of root-knot nematodes and between populations of the same species (Evans and Perry, 2009).

2.5 Reproduction and Development of Root-knot Nematodes

Root-knot nematodes are associated with three types of reproduction, mitotic parthenogenesis (apomixes), meiotic parthenogenesis (automixis) and cross fertilization (amphimixis) (Holterman *etal.*, 2009). Mitotic parthenogenesis is the most common type of reproduction and it is usually occurs in*M. incognita*, *M. javanica* and *M. arenaria* (Castognone-Sereno, 2006). *M. hapla* undergoes facultative meiotic parthenogenetic reproduction (Castognone-Sereno, 2006). Phylogenetic analysis have shown that root-knot nematodes can undergo asexual reproduction through obligate mitotic parthenogenesis (Holterman *etal.*, 2009).

Females of root-knot nematode species lay eggs in gelatinous masses composed of a glycoprotein matrixby the rectal glands (Perry *et al*., 2009). The egg masses are usually found on the surface of galled roots, although they may also be embedded within the

gall tissue. The egg mass is initially soft, sticky and hyaline but becomes firmer and dark brown with age (Perry *et al*., 2009).

Most of the root-knot nematodesspeciesproduce between 50 and 500 eggs, but a few occasionally produce several thousand eggs per female (Agrios 2005; Crow and Dunn, 2005). The juveniles progress through three moulting stages while continuing to feed on the cells of their host plants. When mature, the adult females lay eggs to complete the lifecycle (Westerdahl and Becker, 2011). The optimum soil temperature for root-knot nematode development is 25–28°C. Within this range, the nematode requires three to four weeks to complete itslifecycle. Although they may develop at lower temperatures, the development will take more time (Westerdahl and Becker, 2011). According to Crow and Dunn (2005), the length of reproduction cycle varies considerably depending on nematode species, host plant and temperature of the habitat. When soil temperature is high (above 30^{o}C), root-knot nematodes complete their life cycles in about 30-35 days (Charchar and Santo, 2009).

2.6 Damage Symptoms of Root-knot Nematode Species in Tomato Plant Typical symptoms of root-knot nematode damage can be observedon both aboveground and below-ground plant parts (Noling, 2010). Foliar symptoms of root-knot nematode infestation of roots generally include stunting, premature wilting and slow recovery to improved soil moisture conditions, leaf chlorosis (yellowing) and symptoms characteristic of nitrogen deficiency (Noling, 2010). An increased rate of ethylene production, thought to be largely responsible for symptom expression in tomato, has been shown to be closely associated with root-knot nematode root infestation and gall formation (Noling, 2010).

The most characteristic symptom of the disease which occurs below ground is the presence of galls on the root system (Sikora and Fernandez, 2005). Roots develop multiple small galls; these galls often fuse to cause extensive swelling and distortion of the root system (Kennelly, 2007).Gall size may range from a few spherical swellings to extensive areas of elongated, convoluted, tumorous swellings which result from exposure to multiple and repeated infestation (Noling, 2010).However, the size and the form of the gall depend on the species involved, number of the nematodes in the tissue, and host and plant age (Crow and Dunn, 2005). In tomato, the roots react to the presence of root-knot nematodes by the formation of large, fleshy galls, whereas in most other vegetables, galls are large and firm (Luc *etal.*, 2005b). The roots also become much shorter and bushier than those of healthy plants (Kennelly, 2007). In addition, root-knot nematode infestation in young plants often leads to hooking of the tap root due the presence of females on one side of the cortex (Sikora and Fernandez, 2005).

2.7 Control Methods of Root-knot Nematodes

Due to the prolific reproduction nature and the difficulty in controlling root-knot nematodes, different management techniques are being employed by farmers to mitigate the problem. The strategies used to control root-knot nematodes in the tropics include: nematicides, crop rotation, soil solarization, flooding, biological control and soil amendments such as the use ofmanure, plant parts, composts and wood ash (Barker, 2005).

2.7.1 Use of Synthetic Nematicides in Controlling Root-knot Nematodes To prevent huge crop losses, the control of root-knot nematodes is essential for food security. The use of synthetic chemicals (nematicides) has been the most popular among the farmersin developed countries (Greco, 2009) because some nematicides can quickly control nematodes and prevent significant losses (Noling, 2010). Synthetic chemical control

methods encompass the use of different inorganic compounds in different formulations to kill or interfere with the growth and reproduction cycle of root-knot nematodes in infested fields (Strajnar and Sirca, 2011). The most commonly used chemicals are those that act either as contact nematicides (non-volatile) or as fumigants (volatile) (Strajnar and Sirca, 2011).

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2.7.2 Use of Contact Nematicides (Non-volatile)

Most of the non-fumigant nematicides currently registered for use in vegetables are applied in the soil, however, some can be applied on the foliar, for example, Vydate L (Noling, 2010). These compounds are uniformly incorporated into the soil to enhance contact with the nematodes or in the case of systemics, to facilitate absorption by the plant (Noling, 2010). These nematicides are either oximecarbamate or organophosphate in nature (Strajnar and Sirca, 2011).Common examples of contact nematicides include: aldicarb, cadusafos, calcium hypochlorite, carbofuran, DBCP (1,2-dibromo-3-chloropropane), ethephon, ethopropos, fensulphothion, formalin, isazophos, nemafos, oxamyl and thionazin (Strajnar and Sirca, 2011). Applying these types of nematictdes on established crops, already showing severe damage, is useless and may cause residues in the edible parts of the plants. Their use is suggested when nematode soil population densities at planting are from low to medium (Greco, 2009). The nematicides mentioned above were found to be effective on active stages of the nematodes but not on eggs (Meher *et al*., 2010). Consequently, there can be resurgence of the population from the subsequent generation, thus causing pesticide resistance and total yield loss. Therefore, concern has emergedregardingtheuseofnonfumigants;repeateduse of non-fumigant types of nematicide decreases their efficacy

(Meher *et al*., 2010).

2.7.3 Use of Carbofuran in the Management of Nematodes

Carbofuran® is a broad-spectrum pesticide used in the control of arthropd pests and nematodes (Jada *et al.,* 2010). Carbofuran functions through contact and systemic activity controlling soil and foliar insects and nematodes in many crops. Itwas recommended for the controlof rice water weevil in the USA (Jada *et al.*, 2010). This was the only insecticide that was effective for the control ofthe weevil. To date, rice water weevil resistance to carbofuran has not been documented (Jada *et al.*, 2010). In a separate research, results indicated that application of carbofuran at planting of groundnut significantly reducedthe population of*M. javanica,*compared to the other treatments (Charegani *et al.*, 2012). Also, Senthikumar and Ramakrishnan(2004) reported that application of carbufuran increased the multiplication of *Trichoderma viride* in the soil. The combined application of carbofuran and *Pseudomonas fluorescens* or *Trichoderma viride* significantly improved the growth of okra and reduced the population of *M. incognita*in the soil (Senthikumar and Ramakrishnan 2004).

2.7.4 Use of Fumigants (Volatile Nematicides)

Fumigant nematicides are formulated as liquids which rapidly volatalize to gas and move through open air spaces in soil. Fumigant nematicides include methyl bromide, methyl iodide, chloropicrin, 1,3 dichloropene, dimethyl dibromide and metam sodium and potassium (Noling, 2011).The use of broad-spectrum fumigants effectively reduces nematode populations and increases vegetable crop yields, particularly when compared with non-fumigant nematicides (Noling, 2010). Fumigant treatments are most effective in controlling root-knot nematode when residues of the previous crop are either removed or allowed to decompose. When plant materials have not been allowed to decay, treatment may not be effective in decreasing the populations of root-knot nematodes in soil, particularly when they are in egg stage (Noling, 2010).

Substantial yield may be obtained from crops grown in nematicide-treated soil but, because of the high rate of fecundity and development of root-knot nematodes, populations are rarely totally eliminated by these chemicals (Luc *etal*., 2005b). The general soil sterilant, methyl bromide is extensively used in intensive vegetable production systems to control root-knot nematodes (Crow and Dunn, 2005). However, this chemical has been banned in several countries because of its negative effect on the environment(Noling, 2011). Generally, the most limiting factor in using synthetic nematicides in developing countries is that they are either too expensive, sub-standard or due to lack of technical knowledge. The excessive use and poor handling of these chemicals can lead to health hazard, nematode resistance and destabilization of the ecosystem.

2.7.5 Soil Solarization in Management of Root-knot Nematodes

Soil solarization is a non-chemical technique in which transparent polyethylene tarps are laid over moist soil for a six to 12week period to heat non-cropped soils to temperatures lethal to nematodes and other soil-borne pathogens (Bakr *etal*., 2013). Soil temperatures are magnified by the trapping of incoming solar radiation under the clear, polyethylene panels. To be effective, soils must be wet and maintained at high soil moisture content to increase the susceptibility (thermal sensitivity) of soil-borne pests andthermal conductivity of soil (Noling, 2010). Many types of fertilizers and organic amendments can increase the pesticidal effects of solarization when incorporated in soil prior to heating by releasing biotoxic volatile compounds (Bakr *etal*., 2013).

Certain plants have phenolic compounds which when released in the soil can to kill or repel root-knot nematodes. These plants could be incorporated into the soil and combined with solarization to effectively control root-knot nematodes. For example, the combination of Broccoli residues and solarization proved effective against rootknot

nematodes (Buskov *etal.*, 2002).Solarization significantly reduced the number of juveniles in the soil, number of galls, females and egg-masses of rootknotnematodes(Bakr *etal*., 2013).A reduction of 78 to 100% of root-knot nematodes (*M.javanica* and *M.incognita*) at 15 – 30 cm deep was observed (Buskov *etal.*, 2002).Similarly, Bakr, *etal*. (2013) reported that the highest reduction (95%) in the number of juveniles/250ml soil compared with the control was recorded in the solarized experiment. However, this method could not be effectively carried out in some parts of the subregion due to the fact that the period of the rainy season is short (six months) and taking a month from the season would be a total failure for the farmers. In addition, the price of the polythene sheet is expensive and this might not be easily affordable to the farmers.

2.7.6 Use of Crop Rotation in the Management of Root-knot Nematodes Crop rotation is a very old practice for reducing root-knot nematode infestations. Many root-knot nematodes can reproduce and survive on only specific crop species. Repeated planting of the same crop in the field without interruption will enable any root-knot nematode species to reproduce successfully(Wang and McSorely, 2004). For crop rotation to be effective, crops unsuitable (non-hosts) for nematode infestation, reproduction and development must be introduced in the rotation sequence. In some cases, resistant crop varieties have been used within the rotation sequence to minimize the infestation of some root-knot nematode species.In tomato, a single dominant gene (Mi) has been widely used in plant breeding efforts and varietal development, which has shown resistance to most of the economically important rootknot nematodespecies (Noling, 2010). Also, cowpea (*Vigna unguiculata*(L.) Walp]) is well adapted to cultivation as a cover crop in the tropics, and many cowpea cultivars are poor hosts to root-knot nematodes (Wang and McSorely, 2004).

The roots of marigolds exude chemicals that kill root-knot nematodes (Wang*etal.*, 2007). The cultivation of selected marigold varieties in the rotation consistently resulted in a yield increase of 50% and lowered root-galling by 30% of the subsequently grown tomato(Wang*etal.*, 2007). Groundnut can also be used in rotation with susceptible tomato varieties.According to Mitkowski and Abawi (2011), groundnut is considered to be a good trap crop for *Meloidogyne* spp.

Crop rotation requires careful planning, but, majority of the farmers in the subregion need the necessary knowledge to carefully plan the cropping sequence in the field to avoid excessive build-up of nematodes. In addition, rotations used for nematode management are being altered drastically as a result of the increasing need for food, feed, fibre and more recently, biofuel. Demand and higher prices are affecting crop selection, which then changes the structure of traditional cropping systems and as a result, nematode densities in the soil (Sikora, 2010).

2.7.7 Biological Control of Root-knot Nematodes

Soil microbial communities play an important role in plant health and disease suppression. Beneficial microbes in the microbial communities could promote soil ecosystem health that contributes to suppression of plant pathogens and other pests (Burkett-Cadena *etal*., 2008). Santhosh *et al.* (2005) indicated that tropical soils are rich in beneficial microbes and the biological control potential of the resident microbial fauna and flora is under-exploited. The soil is home to still unknown forms of antagonistic organism that can be used for nematode and disease control.

The most commonly used biological control agents are fungi and bacteria. More than 30 genera and 80 species of fungi are known to parasitize root-knot nematodes(Sun *etal*., 2011). Some fungi use mycelial traps or sticky spores to capture nematodes, for example, *Arthrobotrys* spp. and *Monacrosporium* spp. Other fungi parasitize eggs and

root-knot nematode females, for example, *Pochonia chlamydosporia* (Goddard), *Paecilomyces lilacinus*(Thom.) and *Trichoderma* spp. (Mitkowski and Abawi, 2011).

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood, and other forms of plant organic matter (Verma *etal.,* 2007). They are, for the most part, classified as imperfect fungi, in that they have no known sexual stage (Verma *etal.,* 2007). Rapid growth rate in culture and the production of numerous spores (conidia) that have varying shades of green characterize fungi in this genus (Gams and Bisset, 2002). The reverse side of colonies is often uncoloured, buff, yellow, amber, or yellow-green, and many species produce large quantities of thick-walled spores (chlamydospores) in submerged mycelium (Gams and Bisset, 2002).

The potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930s (Verma *etal*., 2007). This was confirmed by Verma *etal.* (2007) who stated that "different species of *Trichoderma* have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers". Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which primarily contribute to antagonism of these fungi (Verma *etal.*, 2007). Dababat and Sikora (2007) reported that results of field experiments showed a reduction of *M. javanica* population by 36-40% after application of several isolates of *T. viride* (Pers) and *T. koningii* (Oudem).All the *Trichoderma* strains had the ability to colonize *M.javanica* in separated eggs and juvenile experiments (Dababat and Sikora, 2007).

However, the major drawback in using biological agents in nematode control is the inability to economically generate the large amounts of biological material necessary

for application over large areas. Also, the biological agents are very slow in action and in most cases not effective when the nematode population is high.

2.7.7.1 Potential of *T. viride* in the Management of Root-knot Nematodes

T. viride is one of the most commonly reported and widely distributed of all soil fungi. Many physiological, antifungal, and insecticidal activities have been attributed to this species (Domsch *et al*., 2006).*T. viride*represents widely studied fungi that show antigonistic activity towards soil-borne fungal pathogens and nematodes (Domsch *et al*., 2006). The fungus controls pathogens such as nematodes by colonizing the plant roots and providing a physical barrier for the plant against the nematodes, and also enhance the plant‟s root growth and nutrient absorption (Wickramaarachchi and Ranaweera, 2008). Sharon *et al*. (2001) reported that nematicidal activity of *T. viride*may be due to the eggs and larvae being infected through the increase in chitinase and protease activity. As chitin is a major component of egg shell of nematodes, *T. viride* penetrates the eggs, leading to the reduction in population. According to Shebani and Hadavi (2008), direct parasitism of eggs through increase in extra cellular chitinase activity as indicated by egg infection capability and inducing plant defense mechanism, leading to systemic resistance, are the two possible mechanisms for the suppression of nematodes. However, Sharon *et al*. (2001) reported thatvarious mechanisms have been suggested for the biocontrol activity of *T. viride* against plant parasitic-nematodes: antibiosis, parasitism and enzymatic hydrolysis. Enzymes such as chitinase, glucanases and proteases seem to be very important in the parasitism process of the nematode eggs by *T. viride*(Siddiqui and Shaukat, 2004).

2.7.8 Use of Organic Soil Amendments

The European Legislations on environmental protection and human and animal health have revised the utilization of pesticides on agricultural crops, and are now advocating

for research into new alternative control techniques that are environmentally friendly and economically appropriate for users (Scheuer, 2010).

Alternative control techniques, such as organic amendment, have been used with some success (Krueger and McSorley, 2014). There is a strong relationship between soil fertility and plant health, in the sense of the plant‟s ability to resist pests and diseases. Organic amendments can help improve soil structure, and is of great importance to many soil functions, including carbon cycling, raising soil pH and nutrient storage (Krueger and McSorley, 2014).

Also, organic amendments enhance biological activities in the soil and control various plant pathogens, includingroot-knot nematodes(Nagaraju *et al*., 2010). The use of these products for management of root-knot nematodes has been shown in many studies (Oka, 2010). Several types of organic amendments, including plant parts, animal manure and composted materials, have been applied to the soil to suppress populations of root-knot nematodes and enhance crop health and yield (Chang*etal*., 2007).

Some organic amendments, such as plant materials that contain chitin, canrelease ammonia into the soil, suppress plant-parasitic nematode populations directly and enhance growth of microbial antagonists of nematodes (Zasada *et al*., 2003). Nematicidal compounds have been isolated from a great number of plant species (Ferraz and de Freitas, 2004). Neem (*Azadirachta indica* L.) has been widely studied for its nematicidal properties, and has been used as plant extracts, oil cakes, or whole plant materials in a large number of studies, particularly in India (Oka,2010). The application of neem seed cake in the soil was observed to significantly reduce damage (59.5 %) and population of plant-parasitic nematodes (88.6 %) in the soil and roots of cabbage(Zasada *et al*., 2003).

Decomposed products from cruciferous plants have demonstrated good potentials against root-knot nematodes and other soil-borne plant pathogens (Oka *etal*., 2007). During decomposition, these products give out biocidal compounds in the soil, principally, isothiocyanates (Sikora and Fernandez, 2005).The suppression of nematodes by marigold (*Tagetes* spp.) and *Crotalaria* spp. have been much studied. The incorporation of parts of these plants (roots and leaves) into the soil reduced the number of root-knot nematodes on pepper by 85 and 63 %, respectivel (Hooks *etal*., 2010). Oka(2010) indicated that tanninsandphenoliccompounds released from plant residues during decomposition are the active ingredients that kill nematodes. Similarly, Thoden *etal*. (2011) reported that many plant residues and other amendments release nitrogen compounds and organic acids that have adverse effects on nematodes. Ammonia is a common and much-studied by-product of decomposition of organic amendments (Thoden *etal*., 2011). Measured concentrations of ammonia released from compost in pot experiments were found to be above the lethal level needed for *M. javanica*(Treub) suppression (Oka *etal*., 2007).

2.7.9Potential of Cassava Peels in Root-knot Nematode Management

Aqueous extract of cassava peel contains high content of cyanogenic compounds and linamarin (Olabiyi *etal*., 2007). The most important degradable products in cassava peel are acetone cyanohydrin and cyanide. Linamarin was found to be nontoxic to nematodes but the addition of linamarase released acetone cyanohydrin and cyanide which were found to be highly toxic (Olabiyi *etal*., 2007). *In vitro* studies on the effect of cassava cyanogens on second stage juveniles of *M.incognita* showed that both cyanide and acetone cyanohydrin have significant effects and similar mode of action (John *etal.*, 2009). The toxicity was related to the concentration of these compounds and the time of exposure. Highmortality of root-knot nematodes andreduction in root

galling were observed after incorporation of cassava peels into the soil planted with pepper **(**Maina *et al*., 2012**).** Also, Ononuju *et al.* (2014) observed that dry cassava peels significantly reduced the population of root-knot nematodes in the roots and soil and gall index on okra, and was as good as carbofuran in pots.

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2.7.9.1Cyanide Contentin Cassava Peel

Generally, cassava peel contains higher level of cyanide than the pulp (Canadian Food Inspection Agency, 2005). Studies indicated that "bitter" cassava varieties contained an average of 650ppm of the total cyanide in the peels compared to 310ppm in the pulp; the corresponding values for "sweet" varieties were 200ppm and 38ppm respectively (Canadian Food Inspection Agency, 2005). Studies have shown that concentration of cyanide is higher in the fresh cassava peels than the dry peels(Dhas *etal.* (2011). These products are not stable in the environment; therefore, degrade very quickly (Dhas *etal.*, 2011).

2.7.9.2Mode of Action of Cyanogens on Nematodes

Hydrogen cyanide (HCN) is the chemical responsible for tissue hypoxia. In humans, chronic exposure to HCN may cause neurological, respiratory, cardiovascular and thyroid defects (Dhas *etal.*, 2011). The mode of action of cyanogenic glycoside in killing plant-parasitic nematodes is still not clear (Dhas *etal.*, 2011). However, one of the most probable explanations of the biological role of cyanogenic glycosides in some plants is the participation in defense mechanisms against different phytopathogens. Studies have shown that cyanogenic glycosides can act as feeding deterrents or phago-stimulants in insects (Sadasivan and Thyumanavan, 2003).

2. 7.10Potential of Sweet Orange Peels in Root-knot Nematode Management

Citrus peels, commonly regarded as agro-industrial waste, are a source of important

secondary plant metabolites and essential oils (Andrea *etal*., 2003). Citrus peel essential oils are reported to be one of the rich sources of bioactive compounds such as lemonene, coumarins, flavonoids, carotenes, terpenes and linalool. (Mondello *etal*., 2005). The concentration of lemonene was found to be significantly higher in the fresh peel of sweet orange (80.9%),than in the dry peels (66.8%) (Kamal *etal*., 2011). Essential oils from citrus peel have been utilized for their natural antimicrobial and antioxidant properties (Viuda-Martos *etal*., 2008). Generally, it is accepted that biological activities of plant materials are strongly connected to their specific chemical composition, mainly the secondary metabolites such as plant phenolics and flavonoids (Viuda-Martos *etal*., 2008).

Citrus peel extract was found to significantly reduce soil and root population of *M. incognita*(Kamal *etal.*, 2011). Root damage by root-knot nematodes was equally reduced in both treatments in proportion to the levels of application and were significantly different from the control treatment (Kamal *etal.*, 2011). Also, Loumédjinon *etal.* (2007) reported that application of dry peels of sweet orange fruits at 50g/pot were as efficient as the commercial nematicide Rugby10 in reducing nematode population in the roots and soil. In a laboratoryexperiment, the result showed greater nematicidal effect in the extracts of dried pulpified peels of sweet orange with 93.5% mortality of nematodes (Tsai, 2008). Similarly, Mercy *et al*. (2014) observed increase in microorganism activities after application of orange and banana peel powderand extract in pot experiments. In a separate laboratory experiment, Osei *etal*. (2011a) observed that dry peel of sweet orange inhibited hatching of the eggs of *M. incognita* by 93% compared to the control.

2.7.10.1Mode of Action of Lemonene on Nematodes

The mode of action of lemonene (d-lemonene) on nematodes is unknown; however, with insects, it appears to act on the nervous system by effecting leg paralysis followed by convulsions and death (Ntalli *et al*.,2011**)**. Like conventional fumigant nematicides, lemonene exhibits properties of modest water solubility and fumigant action but amenable to application around living plants (Andres *etal*., 2012). However, phytotoxicity has been reported in plant. The concentration level of limonene which can control nematodes may be phytotoxic depending on the sensitivity of the plant(Nikoletta, 2013**)**.

2.7.11Effect of Rice Husk on Nematodes

Rice husk has frequently been utilized in the past for soil improvement (Chun-Yang *etal*., 2006). In most cases, rice husk ash was used as an admixture and stabilizing agent (cement or lime). It has been observed that rice husk ash reduces the plasticity of soils and Maximum Dry Density and increased organic matter content (Chun-Yang *etal*., 2006). In addition, rice husk was observed to have the ability to reduce plantparasitic nematode infestation on crops. According to Prakash and Singh (2014), nematode density and reproduction decreasedprogressively with increasing rice husk amendments. Similar studies done by Hassan *etal*. (2010) showed that amending the soil with organic waste materials such as rice husk and saw dust suppressed the populations of *Meloidogyne* spp. both in the soil and roots of tomato with simultaneous increase in the growth and yield.

2.7.11.1Mode of Action of Rice Husk on Nematodes

The application of rice husk ash has helped to improve soil condition for antagonistic microbial population and better plant growth, thus has enhanced plant resistance to disease pathogens (Halbrendt and LaMondia, 2004). The mode of action of rice husk

on plant-parasitic nematodes is yet to be fully understood, however, Chakrabarti *etal*. (2015) stated that "rice husk in the form of biochar releases certain organic compounds such as phenols and furfurals, which have potential toxic effect on nematodes". These cause the nematodes to under go energy-stress and die. It is assumed that an unidentified chemical trigger exists in the rice husk, which is not destroyed during the hydrothermal carbonization process which causes the stress (Chakrabarti *etal*., 2015).

2. 7.12Effect of Poultry Manure in the Management of Root-knot Nematodes

The application of poultry manure has been observed to have a suppressive effect on root-knot nematodes (Orisajo *etal*., 2007). The mechanisms by which this suppression occurs could be by enhancing the plant resistance through improving the physical, chemical and biological characteristics of the soil (Oka, 2010). Farahat *et al*. (2010) reported that efficacy of poultry manure against plant-parasitic nematodes may either be due to stimulation of specific micro-organisms that were capable of parasitizing eggs and juveniles or production of substances from decomposition of the manure which were toxic to the nematodes. According to Meyer *etal*. (2011), poultry litter compost extracts inhibited hatch of *M. incognita* eggs and immobilized juveniles, indicating that chemical compounds might account for some of the mode of action of the compost against the nematodes. Previous studies revealed significant reduction in number of root-knot nematodes in the roots and soil of tomato due to ammonia concentration in poultry manure (Oka *etal*., 2007). However, some controversy still remains about the reliability of poultry manure for nematode management. Intensive studies on poultry manure in Europe andothertemperate countries showed an increase in the number plant-parasitic nematode with addition of poultry manure (Thoden *etal*., 2011).

2.7.12.1Effect of Soil Nutrients and pH on Root-knot Nematodes

There is a strong relationship between soil fertility and plant health, in the sense of the plant‟s ability to resist pests and diseases (Akhtar and Malik, 2000). In general, nutrients can directly or indirectly predispose plants to pathogen attack. The types of nutrients and levels in the soil can reduce or increase disease severity through influencing the resistance or tolerance of the host plant (Agrios, 2005). Application of fertilizer especially at the right quantity can, partially, offset nematode-induced damage by stimulating plant development (Ferraz *et al*., 2010). Application of adequate plant nutrient with potassium reduces disease severity due to increased resistance to the penetration and development of pathogens (Hurchanik*et al*., 2003). Dias-Arieira *et al.* (2012) reported that potassium phosphite was effective in reducing the number of *Pratylenchus.brachyurus*in maize. Oka *etal*. (2007) also reported a reduction in the number of *Heterodera avenae* (Wollen) and *Meloidogyne marylandi* (Jepson and Golden) in wheat and oats after aerial application of potassium phosphite.

This result is attributable to phosphite‟s ability to translocate along the plant‟s xylem and phloem (Wang and Bergeson, 2004).

Nitrogen is another nutrient element in the soil which plays significant role in the management of root-knot nematodes (Wang and Bergeson, 2004). However, the form in which the nutrient is available, whether ammonium (NH_4^+) or nitrate (NO_3^-), has more effect on the severity of the nematode attack than the quantity of nitrogen available (Ferraz *et al*., 2010). Nitrogen in the form of ammonium, present in fertilizers and organic matter, is more harmful to nematodes than in nitrate form (Oka *etal*., 2007)). The nematicidal property of ammonia is mainly attributable to its plasmolytic effect around the point at which it is applied to the soil (Oka *etal*., 2007). The suppressive effect of ammonium on nematodes largely depends on its level of concentration in the soil (Renco *etal.*, 2010).

Plant-parasitic nematodes can survive in soils over wide ranges of pH. In the case of root-knot nematodes, the effect of soil pH varies greatly (Esfahani, 2009). According to Luc *et al*., (2005a),*Meloidogyne* species survive and reproduce at pH levels ranging from 4.0 to 8.0. Emergence of *M. javanica* was found to be greatest between 6.4 and 7.0 (Luc *et al*., 2005a) and inhibited below pH 5.2 (Asif *et al*., 2015). However, many tropical soils are very acidic (pH of 4.5) but this does not seem to prevent the population build-up of root-knot nematodes (Asif *et al.*, 2015).

2.8 Identification of Root-knot Nematodes

Correct identification of root-knot nematodes is a prerequisite for effective and efficient control. Most species that infest plants cultivated in the subregion are closely related such as *M. incognita, M. javanica* and *M.arenaria*, and are therefore collectively referred to as the *M. incognita* group (MIG) (Hunt and Handoo, 2009). Verification of mixed populations and detection of species require use of identification techniques that are accurate. In this part of the continent, identification of root-knot nematodes is limited by inadequate expertise and equipment for the laboratories. Nonetheless, different techniques have been devised to improve the existing ones in order to help attain accurate identification. Identification methods of root-knot nematodes are based on either morphological, biochemical and/or molecular techniques. In Ghana, Kwara *etal*. (2014) identified *M. incognita, M. javanica* and *M.arenaria* in some vegetables, using esterase and malate dehydrogenase isozyme (MDH) method.

2.8.1 Morphological Identification

Root-knot nematodes (*Meloidogyne* spp.) are usually identified using morphological features and morphometrics on second-stage juveniles (J2), males and perineal patterns

of mature females (Hunt and Handoo, 2009). Great similarities exist in the basic morphology of root-knot nematode species. However, some distinctive structures are significant in the identification of tropical species. These body features include morphology of perineal patterns, length of the stylet, body width, body length, the head morphology of females, males, and second-stage juveniles (Hunt and Handoo, 2009).

With the growing number of identified species in the genus *Meloidogyne*, it is imperative that species description conforms with general standard in order to facilitate accurate comparisons and differential diagnoses (Perry *etal.*, 2009). The morphological details of these species are important for identification and for identifying phylogenetic relationships (Karssen and Moens, 2006). In addition, these morphological details are often used to determine physiological function (Shepherd and Clark, 1983). Morphological observations are useful in understanding the relationship of the environment with the nematode (Perry *etal*, 2009), and also give perception into the relationship of host-parasite interaction (Karssen and Moens, 2006); this relationship may have an influence on nematode morphology (Perry *etal.*, 2009).

Although morphology of juveniles can provide a relatively reliable assessment for species assignation (Karssen, 2005), species-level identification, in practice, is complicated by genetic, climatic and anthropogenic factors associated with the dynamic nature and global scope of present-day agricultural production (Hunt and Handoo, 2009). In other words, there is no guarantee that an agricultural field contains only a single species of *Meloidogyne* or that the diagnostic descriptions currently available cover all of the diversity in the genus and will permit reliable identification(Perry *etal.*,

2009). Therefore, in order to have accurate identification, other options such as biochemical and molecular methods are used.

2.8.2 BiochemicalMethod

2.8.2.1 Identification of Root-knot Nematodes usingIsozyme

Nematode systematics no longer rely exclusively on the classical approach to taxonomy based on morphological features and, since the 1970s, experts have increasingly incorporated diagnostic tests based on biochemical identification to meet the need for rapid and accurate diagnostics (Perry and Moens, 2006). Isozyme electrophoretic profiles, often using esterase and malate dehydrogenase, have been recognized for a number of root-knot nematode species and can provide a useful, routine diagnostic test, particularly for morphologically variable species like *M. incognita* and *M. javanica*(Hernández *etal*., 2004). Zu *etal*. (2004) observed 46 populations from 14 provinces in China and found five esterase phenotypes.The relative stability of the isozyme phenotypes within *Meloidogyne* species (De Waele and Elsen, 2007) makes it an attractive method, although there are some problems. The prevalence of intraspecific variants and the difficulty in determining size variants between species (for example the esterases of *M. incognita* and *M. hapla*) have necessitated the use of more than one enzyme system to confirm the identity of some isolates(De Waele and Elsen, 2007). Based on these bottlenecks, nematologists prefer other methods such as molecular analysis to izozyme.

2.8.3 Molecular Identification

Molecular methods based on DNA can be used in all the stages of the nematode life cycle, and they are fast and reliable. There are many approaches such as Random Amplified Polymorphic (RAPD), Sequence Characterized Amplified Region (SCAR), Restriction Fragment Length Polymorphisms (RFLPs) and Internal Transcribe Spacer

(ITS) variation to name a few. The ribosomal DNA (rDNA) repeating units, including 18S, 28S, and 5.8S coding genes and the ITS regions have been widely used for phylogenetic studies (Adam *et al*., 2007). The ITS regions are probably the most extensively used genetic markers among living organisms and the most common species-level marker used for plants, protists and fungi (Hajibabaei *et al*., 2007).The multi-copy basis of rDNA provides ample target for PCR amplification, and sufficient variation and stability occurs within it for reliable discrimination of most species (Adam *et al*., 2007). For example, PCR−RFLP of the ITS regions have been used to identify *M. incognita, M. javanica*, *M. hapla*, *M. chitwoodi, M. fallax*(Zijlstra *et al*., 2000). In addition, the ITS–RFLP approach, has been used to determine the composition of species in mixtures by comparing the intensity of bands produced for each species. Therefore, based on the advantages outlined, it was appropriate to use ITS–RFLP approach in this study to identify the*Meloidogyne* species.

CHAPTER THREE

3.0PRODUCTION PRACTICES AND PEST AND DISEASE PROBLEMS OF TOMATOESIN ASHANTI REGION OF GHANA

3.1Introduction

Tomato (*Solanum lycopersicum* L.), is one of the important vegetables commonly grown by smallholder farmers in Ghana (Osei *etal.*, 2010). The daily consumption of tomato in the form of paste, sauce and ketch-up is higher than other vegetables (Asare-Bediako *etal*., 2007). It is rich in nutrients such as vitamins and minerals which are important to well-balanced human diets. It is also an essential dietary component because it contains high level of lycopene, an antioxidant that reduces the risks related to several cancer diseases (Srinivasan, 2010).

The government of Ghana is encouraging the development of the tomato industry in order to diversify the country‟s export base (GIPC, 2013). However, yields obtained by farmers are far below the potential yield of the crop.In 1998, the average yield of tomato was estimated at 13.5T/ha(Wolff, 1999) and in 2010 it was estimated at 7.5T/ha (MoFA, 2012). This has created a yield gap of about 50%. One of the main constraints associated with the low yield are the problems of pests and diseases (AduDapaah and Oppong-Konadu, 2002). Similarly,Anang *et al*. (2013) attributed the low yield of tomato at farmers‟ fields mainly to the high incidence of insect pests and diseases.

Previous surveys carried out in the Savanna and Forest-Savanna Transitional zones by Adu-Dapaah and Oppong-Konadu (2002) showed that foliar and soil-borne diseases such as damping-off, blight, Fusarium wilt and Tomato Mosaic Virus were the most economically important on tomato in Ashanti Region. Similar study by AsareBediako and Micah (2014) found insect pests such as caterpillars and whitefly to be the most economically important on tomato in Ghana. In furtherance to these pests and diseases problems, the current study looked at other soil borne pathogen such as root-knot nematodes and their management strategies. Therefore, it was necessary to ascertain farmers‟ tomato production system in Ashanti Region, and identify pest and disease constraints and their management strategies.

3.3 Methodology

Ashanti Region of Ghana lies between longitudes 0.15W and 2.25W, and latitudes 5.50N and 7.46N. The region shares boundaries with Brong-Ahafo Region in the North, Eastern Region in the East, Central Region in the South and Western Region in the South West. It covers a land area of 24,389 square kilometers representing 10.2% of the total land area of the country (MoFA, 2012).

The region has bimodal rainfall pattern, with highest amount in May/June and October for the major and minor seasons, respectively. Mean annual rainfall is between 1100 and 1800 mm. The average annual temperature is 25.5°C in the southern parts of the region and 32°C in the northern part. The mean yearly humidity is about 85% in the southern parts of the region and 65% in the northern part (MoFA, 2013).The vegetation cover is moist Semi-deciduous Forest in the southern part of the region, whilst the Guinea Savanna occupies the northern part, which consistsof short deciduous and fire tolerant trees. Also, riverine forests occur along the Afram River and streams of the Savanna zone (MoFA, 2012). Soils in Ashanti Region are mainly Acrisols. Tomato production is an important farming activity in the region (MoFA,
2013).

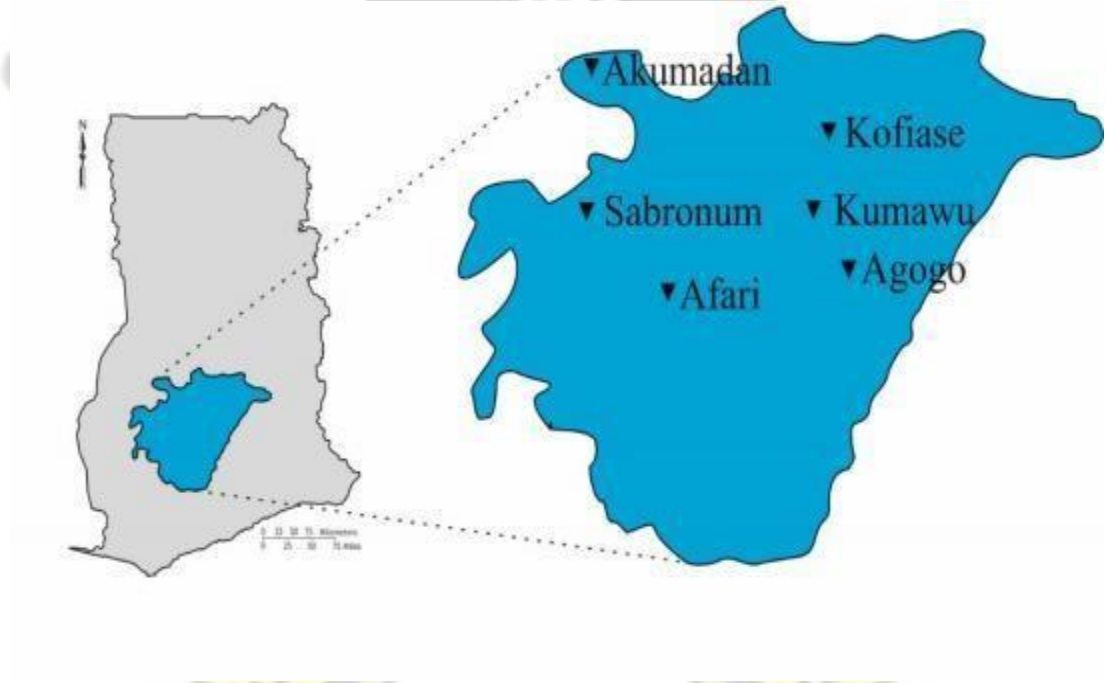

Figure 3.1 Study sites in Ashanti Region of Ghana

3.4 Data Collection and Analysis

The survey was conducted in March, 2015. Multistage sampling technique was used to select the farmers. The study started with purposive selection of the surveyed sites in

consultation with the Ministry of Food and Agriculture in the region. A total of six sites, namely Akumadan, Agogo, Sabronum, Kumawu, Afari and Kofiase (Fig.1) were selected. In the second stage of sampling, the names of tomato farmers were listed in the households. Twenty tomato farmers were randomly selected from each site using lottery method. Farmers were interviewed individually using both close-and open-ended questionnaires. Detailed information on crop production practices, pests and diseases and their management strategies were collected. The data were analyzed using Statistical Package for Social Science (SPSS) for Windows. Descriptive statistics were used and means were presented using tables and graphs.

3.5 Results

3.5.1 Production practices

The tomato varieties cultivated by the respondents were: Power, Pectomech, Pectofake, Akoma and Rhino. Power was found to be the most popular variety with 40% of the respondents cultivating it, followed by Pectomech and Pectofake with 20% each. Land area used by respondents for tomato cultivation in the surveyed sites varied from 0.2 to 6ha. Higher percentage (72%) of the respondents had farm sizes ranging from 1.6 to 2.0ha. Majority of the respondents (85%) cultivated tomato in March/April in the major season, while in the minor season most of the respondents (90%) cultivated in September (Table 3.1). In the case of land preparation, all the respondents planted tomato on ridges and mentioned that they applied fertilizer to increase the fertility of the soil (Table 3.1).

Table 3.1Production Practices Carried out by Tomato Farmers in the Surveyed Area

Activity	Frequency					Total
	Percent respondents					
grown	40	20	20	10	10	**100**
Tomato variety	Power	Pectomech	Pectofake	Akoma	Rhino	
Land Area cultivated to tomato (ha)	0.2 – 0.4	0.8-1.2	1.6-2.0	2.4-2.8	3.2-3.6	
	2	20	72	4	2	**100**
Soil preparation method	Ridging	Ploughing	Zero tillage			
	100	0	0			**100**
	Major season			Minor season		
Period of growing tomato	March-April	May-June		September	October	
	85	15	**100**	90	10	**100**
Type of fertilizer applied	NPK and urea	NPK and ammonium sulphate	NPK and Sidalco foliar fertilizer	NPK alone		
	15	70	10	5		**100**
Amount of fertilizer used	1bag NPK and ½ bag/ha Ammonium Sulphate	2bags NPK and 1bag/ha Ammonium Sulphate	3 bags NPK and 1 bag/ha Ammonium Sulphate			
	5				70	25 **100**
Total (%)						

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Seventy percent (70%) of the respondents applied NPK (15:15:15) and ammonium sulphate $(NH_4)_2SO_4$ fertilizers, followed by NPK and urea (15%), and then NPK and Sidalco NPK foliar fertilizer (10%). About 5% of the respondents applied NPK alone. Majority of the respondents (70%) used two bags (50kg each) of NPK and one bag (50kg each) of $(NH_4)_2SO_4$ per hectare, while 25 and 5% of the respondents applied three bags of NPK and one bag of $(NH_4)_2SO_4$ and one bag of NPK and half a bag of $(NH_4)_2SO_4$ per hectare, respectively (Table 3.1).

3.5.2 Pests and Diseases Associated with Tomato Production in the Ashanti Region

The pests and diseases mentioned by the respondents in the survey were: root-knot nematodes, fruit borers, whiteflies, mites, damping-off, wilting and leaf-curl (Table 3.2).

Table 3.2 Pests and Diseases Associated with Tomato Production by the Respondents in the Surveyed Area

Activity	**Percent respondents**						**Total (%)**
	Pests				Diseases		
Type of pests and diseases reported	Root-knot nematode	Fruit borers (caterpillars)	Whitefly	Mites	Dampingoff and wilting	Leaf curl	
Total (%)	30	25	20	5	10	10	**100**

Most of the respondents (30%) mentioned root-knot nematodes as the major pest affecting tomato, followed by fruit borers (25%), whiteflies (20%) and mites (5%). Damping-off and wilting (10%) and leaf curl (10%) were also observed by the respondents as fungal and viral disease problems in tomato cultivation (Table3.2).

3.6 Management Practices Carried out by Respondents

For the management of tomato pests and diseases, the respondents indicated the use of either insecticides and fungicides combined or insecticides alone. Ninety-five percent (95%) of the respondents applied insecticide and fungicide as mixture, while 5% used insecticides alone to control pests and diseases. None of the respondents used nematicide or fungicide alone to control pests and diseases (Table 3.3).

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Table 3.3 Management Practices Carried out by the Respondents in the Surveyed Areas

	Percent respondents				
Management practice used	Insecticide and fungicide	Insecticide alone	Fungicide alone	Nematicide	**Total (%)**
	95	5	0	0	**100**
Total (%)					
Other management practices	Crop rotation and weeding	Botanical Extracts	Organic amendments		
Total (%)	100	0	0		**100**

Apart from application of synthetic pesticides, farmers used management methods such as crop rotation and weeding. All the respondents reported that they practiced crop rotation and weeding to also control pests and diseases. None of the respondents applied plant extracts or organic amendments as pests and diseases control strategy (Table 3.3).

3.6.1 Insecticides and Fungicides Used in the Management of Pests and Diseases on Tomato by the Farmers in the Surveyed Areas

The result showed that the farmers used mainly insecticides and fungicides to control pests and diseases on tomato (Table 3.4).

Table 3.4 Insecticides and Fungicides Used by Farmers to Control Pests and Diseases on Tomato

Pesticidetype/ Trade name				
Fungicides	**Active Ingredient**	**Manufacturer**	**Country**	**Percent respondents**
Dithane M-45	Mancozeb	Dow Chemical Company LLC	China	95.0
Kocide	Cupric hydroxide	Certis Company	United States of America	5.0
Total (%)				**100.0**
Insecticides	**Active Ingredient**	**Manufacturer**		**% Frequency**
Golan	Acetamiprid	ADAM Agricultural Solution Ltd	Israel	4.5
Attack	Emamectin benzoate	Anchor Allied Factory Ltd.	United Arab Emirates	38.6
Lambda	Lambda cyhalothrin	Syngenta Group Company	China and India	28.8
Confidor	Imidacloprid	Bayer	China	4.1
Control	Emamectin benzoate	Anchor Allied Factory Ltd.	United Arab Emirates	2.7
Karate	Lambda cyhalothrin	Syngenta Crop Protection AG	Switzerland	15.4
Combat	Lambda cyhalothrin	Aceto Agricultural Chemical Corporation	United States of America	2.7
Dursban	Chlorpyrifos ethyl	Shenzhen Yufull Industry Company Limited	China	3.2
Total (%)				**100.0**

Most of the respondents(95%) used Dithane M-45 to control diseases on tomato while the rest used Kocide (5%). For the insecticides, majority of the respondents (38.6%) used Attack followed by Lamda and Karate with 28.8 and 15.4%, respectively. Other insecticides used by the respondents were Golan (4.5%), Confidor (4.1%), Control

(2.7%), Dursban (3.2%) and Combat (2.7%) (Table 3.4).

3.6.2 Crop Rotation Systems Practiced by Respondents in the Surveyed Areas

There were ten crop rotation systems outlined by the respondents during the survey (Fig. 3.2).

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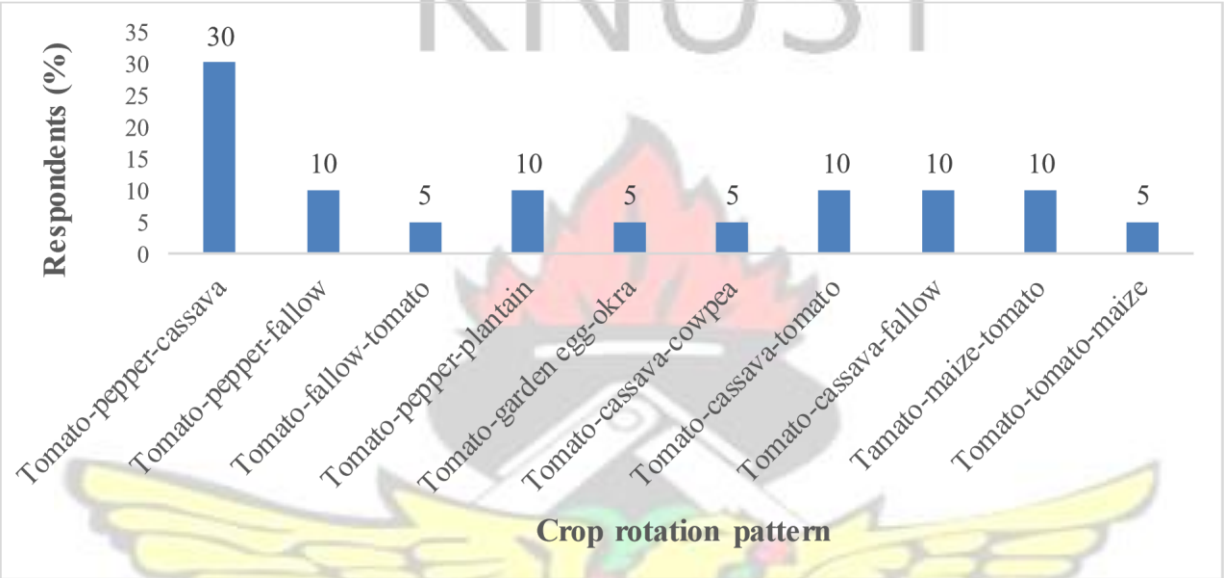

Figure 3.2 Crop Rotation Pattern Practiced by Respondents as Root-knot Nematode Management Strategy

The most predominant system (30%) practiced by the respondents in a threerotational cycle was tomato, followed by pepper, and then, cassava. The other systems practiced were: tomato-pepper-fallow, tomato-pepper-plantain, tomatocassava-tomato, tomato-cassava-fallow and tomato-maize-tomato with 10% each (Fig. 3.2). Five percent (5%) of the respondents practiced tomato-fallow-tomato, tomato-garden egg-okra, tomato-cassava-cowpea and tomato-tomato-maize.

Table 3.5 Length of Fallow Period Practiced by Tomato Farmers in the Surveyed Areas

Period of fallow	Percent respondents/year(s)			Total (%)
	< 2 years	2 _ 3 years	4 _ 5 years	
Total (%)	20	70	10	**100**

The majority of the respondents (70%) practiced two to three years fallow period, followed by less than two years (20%). Ten percent (10%) of the respondents practiced fallow periods between 4 and 5 years (Table 3.5).

3.6.3 Effectiveness of management practices of tomato farmers in the surveyed areas

The effectiveness of management practices by the respondents against insect pests, diseases and root-knot nematodes is presented in Table 3.6. All the respondents agreed that the management practices against insect pests and diseases were effective. However, 85% of the respondents considered their management practices less effective against root-knot nematodes, while 15% considered itineffective. None of the respondents considered their management practices against root-knot nematodeseffective (Table 3.6).

Table 3.6 Effectiveness of Pest and Disease Management Methods by the Respondents in the Surveyed Areas

Pests and diseases management practices	Percent respondents			Total (%)
	Not effective	Less effective	Effective	
Insecticides against insect pests	0	0	100	**100**
Fungicides against diseases	0	0	100	**100**
Crop rotation and weeding against root-knot nematodes	15	85	0	**100**

3.7 Discussion

Most of the respondents planted tomato in March(major season) and in September(minor season). According to respondents, this was done at the onset of the rains to enable them harvestearly for good market price. This agrees with the finding of Adu-Dapaah and Oppong-Konadu (2002) who reported that majority of farmers who grow tomato in the major season planted from mid-March to April, while that of the minor season was done around September.

The results revealed that Power tomato variety was the most cultivated in the surveyed areas. This is in line with Robinson and Kolavalli (2010) who reported that Power variety was widely used by most of the farmers under rainfed conditions in Ghana.The landarea used by the respondents for tomato production varied in size among the farmers. This result is consistent with Asare-Bediako and Micah (2014) who stated that farm sizes of the majority of vegetable farmers in Ghana ranged from less than 0.4 to 4ha. Also, Aidoo *etal*. (2014) indicated that, the farmers cultivate tomato on farm sizes of 2.0ha on the average. This shows that tomato cultivation is done by smallholder farmers as reportedby the Ministry of Food and Agriculture

(2011).

The farmers applied mainly chemical fertilizers to improve soil fertility and most of the respondents used NPK and $(NH_4)_2SO_4$ as basal and topdressing, respectively. However,few of them applied NPK alone after transplanting. Different rates of the fertilizers were used by the farmers on tomato. Donkoh *etal*. (2013) observed intensive use of inorganic fertilizer by tomato farmers in northern Ghana.

In the case of pests and diseases, root-knot nematodes were indicated by most of the respondents as the major pests infesting tomato in the surveyed area. The majority of the respondentssaid that they were aware of the damage caused by the nematodesfrom the trainings received from the Ministry of Food and Agriculture or from other farmers.

This observation agrees with findings of Asare-Bediako and Micah (2014) who found root-knot nematodes as the major pests of tomato in Ashanti Region. The authors further indicated that other pests frequently encountered and described by the farmers were insect larvae (caterpillars) and whitefly.

The result on diseases from this study is in line with that of Adu-Dapaah and Oppong-Konadu (2002) who found damping-off and wilt as economically important constraints for tomato production in Ashanti Region.

Pesticides were observed to be widely used by farmers in the study areas. All the farmers interviewed applied insecticides to control pests on tomato. Heavy use of insecticides by farmers can be explained by the economic importance of fruit borers, caterpillars and whiteflies on tomato. This is in agreement with Owusu-Boateng and Amuzu (2013) who observed over-reliance on synthetic insecticides in the management of pests and diseases by vegetable growers in Ghana. Similarly, AsareBediako and Micah (2014) stated that only few of the respondents used pesticides for disease control, indicating that pests, but not diseases, are the major limiting factors to the production of various vegetables. None of the respondents mentioned the use of nematicides during the surveys. This is in agreement with Asare-Bediako and Micah (2014) who reported that the types of pesticides commonly used by vegetable farmers were insecticides (61.7%), followed by fungicides (32.7%) and herbicides (5.5%).

All the respondents reported that they practiced crop rotation as management method to control pests and diseases particularly, root-knot nematodes. The most common cropping system outlined by majority of respondents was tomato-pepper-cassava in sequential order. Similar result was obtained by Asare-Bediako and Micah (2014) who indicated that farmers cultivated pepper after tomato in a three-rotational cycle. This type of crop-rotational cycle can have negative impact on the second crop (pepper) as

the first crop (tomato) may be susceptible and favour root-knot nematode build-up. The initial inoculum in pepper will be so high that the desired output might not be obtained due to serious nematode damage to the crop. For crop rotation to be effective, crops unsuitable (eg. Groundnut and beans) for nematode infestation, reproduction and development must be introduced in the rotation system. According to Noling (2010),repeated planting of the same crop or crops in the field without interruption will enable some root-knot nematode species to reproduce successfully. Damage caused by *Meloidogyne* spp. is related to their population densities in soils at planting and their reproduction potential (Wesemael amd Moens, 2008).

3.8 Conclusions and Recommendations

Power variety was the commonest tomato variety cultivated in the surveyed areas. Planting of tomato was done at the onset of the rains in the major and minor seasons. The farm sizes were generally small and chemical fertilizers were mainly used as soil amendment. The knowledge of tomato farmers in determining the damage of rootknot nematodes was high. Root-knot nematodes were observed as the most important pest in tomato production, followed by fruit borers and whiteflies. In general, the farmers used pesticides such as Attack, Lambda and Karate to control insect pests, while Dithane M-45 and Kocide were used to control fungal pathogens. Many of the tomato farmers also practiced crop rotation to reduce the severity of pests and diseases. The respondents indicated that management methods against root-knot nematodes were less effective. However,the insecticides and fungicides were found to be effective against insect pests and fungal disease pathogens in the surveyed areas.

Tomato farmers should be trained on crop rotation systems that are effective in reducing pests and diseases incidence and severity on tomato plant. The Ministry of Food and Agriculture should organize training for tomato farmers on pesticide usage, safety and

disposal of pesticide containers, since it is the main current means of control for insect pests and diseases on tomato.

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CHAPTER FOUR

4.0 EFFICACY OF ORANGE AND CASSAVA PEELS IN THE MANAGEMENT OF ROOT-KNOT NEMATODES*IN VITRO* AND *IN VIVO*

4.1Introduction

Root-knot nematodesare among the most polyphagous and damaging of plant-parasitic nematodes (Elling, 2013). In the tropics, significant yield losses have been recorded on tomato due to root-knot nematode damage and, in some cases, the plants die before reaching maturity (Singh and Khurma, 2007). The high rate of development and fecundity of these nematodes, make them difficult to control on crops (Crow and Dunn, 2005).

In many cases, crop losses are reduced by the annual application of expensive and highly toxic soil fumigants or non-fumigant nematicides. These chemicals pose serious human and environmental health hazards and, therefore, not sustainable. In addition, the economic cost of research and registration of new chemicals is a big obstacle for prospective new chemical nematicides to overcome.Also, agrochemical companies are more likely to focus their spending on research into products with a potentially high market-value such as herbicides and insecticides, rather than nematicides. Therefore, many nematologists are pessimistic about the importance of future chemical management of nematodes.Consequently, several groups of nematologists are trying to develop plant-based chemical products for nematode management.

Alternative control techniques, such as soil organic amendment have, been used with levels of some successes. The use of organic amendments for management of plantparasitic nematodes has been demonstrated in a large number of studies (Oka, 2010).

Neem seed cake, castor seed cake and castor bean extract have been widely studied for their nematicidal properties (Oka, 2010; Ribiero and Lima, 2012; Adomako and Kwoseh, 2013).

Sweet orange and cassava are largely consumed in Ghana but most of the peels end up as wastes (Seidu *et al*, 2012) which cause both economic and environmental problems as waste. The effective and sustainable use of sweet orange and cassava peels wastes as organic amendment for root-knot nematode management is highly desirable. Loumédjinon *etal.* (2007) reported significant reduction of root-knot nematode in roots and soil of carrot, using dry orange and cassava peels in the Republic of Benin. However, limited research has been conducted on sweet orange and cassava peels and their combination as organic amendments to manage root-knot nematodes.

In Ghana, there is limited information on the use of plant materials in the management of plant-parasitic nematodes (Osei *etal*., 2011a). Farmers mostly depend on cultural practices such as crop rotationwhich sometimes do not give good result.The purpose of this study was not only to evaluate the efficacy of fresh and dry peels of sweet orange and cassava as soil amendemnet materials in the management of root-knot nematodes and their effect on fruit yield, but also to evaluate the effect of sweet orange and cassava peels on the biocontrol fungi (*Trichoderma* spp.) and freeliving nematodes.

4.2Experiment 1: Evaluation of aqueous extracts ofsweet orange and cassava peel for their nematicidal potentials on root-knot nematodes *in vitro*

Root-knot nematodes are widely distributed and they parasitize nearly every species of vegetable in Ghana. These pests cause serious constraint to vegetable production.

Majority of tomato farmers practice crop rotation to manage root-knot nematodes. However, this system requires careful planning of the cropping sequence to avoid population build-up. Organic amendments have been widely used for the management

of plant-parasitic nematodes. Research on the use of organic amendment materials such as neem seed cake and castor seed extracts yielded good results elsewhere. Therefore, the objective of this experiment wasto evaluate the efficacy of some selected organic amendments in the management of root-knot nematodes*in vitro* and *in vivo*.

4.3Materials and Methods

4.3.1 Location of the Experiment

The study was carried out in the Nematology laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, from March, 2014 to December, 2015.

4.3.2 Experimental Design

The experiment was arranged in Completely Randomized Design (CRD) with five replications of seven treatments.

The treatments used were aqueous extracts of:

1. Fresh orange peel
2. Dry orange peel
3. Fresh cassava peel
4. Dry cassava peel
5. Poultry manure
6. Rice husk
7. Sterilized water was used as control/standard

4.3.3 Sources of the Organic Amendment Materials

Each of the organic materials was collected from the same source to ensure homogeneity of the materials.Fully riped fruits of the sweet orange variety, Valencia (*Citrus sinensis* [L] Obsbeck) were collected from the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI), Fumesua, Kumasi, Ghana.

The cassava variety “Debo” was obtained from a farmer‟s field in Kumasi. Rice husk of‟La pet” variety was collected from a dehulling plant in Kumasi. The poultry waste was also collected from Attoh‟spoultry farmin Kumasi. The manure was a mixture of saw-dust used as litter and birds dropping.

4.3.4 Analysis ofNutrient Content of the Organic Amendments

Samples were collected from each organic amendment and taken to the Soil Science laboratory of the Department of Crop and Soil Sciences, KNUST, for nutrient analysis. All the samples were dried in the oven at 70°C for three days before processing. The samples were ground into powder, using a grinding machine ((Micor doughLAB, Perten Instrument Group, Canada)before the following analysis were carried out:

4.3.4.1 Determination of pH of the Organic Amendments

A weight of 5g of oven-dried powder of each organic amendment was placed in 50ml Erlenmeyer flask. Twenty-five millilitres (25ml) of distilled water was added and the suspension was stirredvigorously, using a stirring rod for 10min. The suspension was allowed to stand for 30minbefore reading. The pH meter was calibrated using a blank at pH of 7.0 and 4.0, respectively. The electrode of the pH meter was inserted into the partly settled suspension in the beaker and the number on the pH meter was recorded. Each sample was taken three times and the mean was calculated.

4.3.4.2 Preparation and Dry Ash Digestion of Organic Amendments for Elemental Analysis

One gramme (1g) of each sample was weighed into a clean ceramic crucible separately. The samples were arranged in a muffle furnace with a temperature of 500^{o}C for a period of 2h. The samples were allowed to cool down in the furnace. Samples were then removed from the furnace and transferred into 50ml centrifuge

tubes. All the tubes were properly labelled before placing into the centrifuge. The crucibles were rinsed with 10ml of distilled water into the centrifuge tubes. More rinsing of the crucible with 10ml of aqua regia was done. The samples were agitated for 5min on a mechanical reciprocating shaker. At the end of the shaking, samples were centrifuged for 10min at 3000rpm and then transferred into 100ml volumetric flask. The clear supernatant digest was decanted into clean reagent bottles for the determination of nitrogen (N), phosphorus (P), calcium (Ca), magnesuim (Mg), sodium (Na) and potassium (K) for each amendment.

4.3.4.3 Determination of Phosphorus (P)

During this process, a vanadomolybdate reagent was prepared by dissolving 22.5g of ammonium molybdate in 400ml of distilled water and 1.25g of ammonium vanadate in 300ml of boiling distilled water. The vanadate solution was added to the molybdate solution and cooled to room temperature. Two hundred and fifty millilitres (250ml) of analytical grade nitric acid was added to the solution mixture and diluted to 1l with deionized water. The standard phosphate solution was also prepared by dissolving 0.2195g of analytical grade Potassium dihydrogen phosphate(contained 50µg P/ml) in 1000ml distilled water. A standard curve was prepared by pipetting 1, 2, 3, 4, 5 and 10ml of standard solution (50µg P/ml) in 50ml volumetric flasks. Ten millilitres (10ml) of vanadomolybdate reagent was added to each flask and the volume made up to 50ml with distilled water. Five millilitres (5ml) of the sample solution was placed in 50ml volumetric flask. Ten millilitres (10ml) of vanadomolybdate reagent was added. The sample was kept for 30min for colour development. A stable yellow colour was developed. The sample was read on the colorimeter at 430nm. The observed absorbance was used to determine the P content from the standard curve. The % P was

calculated using the (FAO, 2008) formula:

$$P\ (mgkg^{-1}) = \frac{Graphreading x\ 25}{w x\ 1000}$$

Where:

w = sample weight in grammes

25= ml of final sample solution

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4.3.4.4 Determination of Potassium (K) and Sodium (Na)

Analytical grade potassium chloride and sodium chloride, weighing 1.908g and 2.542grespectively,and previously dried in an oven for 4 hours at 105^{o}C, were each dissolved separately in 200ml of deionised water. The two solutions were mixed together and the volume topped up to 1000ml. This gave a combined standard of 1000ppm. For K, a calibration curve (standard curve) of 200, 400, 600 and 800ppm was prepared. Also, a standard curve of 20, 40, 60 and 80ppm was prepared for sodium. The emission values were noted, using the flame photometer. The standard curves were obtained by plotting emission value against their respective concentrations (FAO, 2008). Calculation:

$$\%\ K = \frac{\text{Graph reading}}{\text{w x 100}}$$

Where:

W = weight of sample used

4.3.4.5 Determination of calcium (Ca) and magnesium (Mg)

Calcium and magnesium were determined by EDTA titration (IITA,1979). The reagents used were prepared as follows:

Buffer solution – A 60g of ammonium chloride was dissolved in 200ml of distilled water. Five hundred and seventy millilitres (570ml) of concentrated ammonium hydroxide was added and diluted to 1000ml in a volumetric flask.

Potassium cyanide: A 10% KCN (W/V) was obtained by dissolving 50g of KCN in 500ml of distilled water in a volumetric flask.

Potassium hydroxide: A 10% KOH (W/V) was obtained by dissolving 100g of KOH in a litre of distilled water.

Calcone – red (cal – red) indicator: This indicator gives red coloration when Ca^{2+} is absent but gives bluish colour when Ca^{2+} is present.

Triethanolamine (TEA): A 30% (V/V) was obtained by diluting 300ml TEA in a litre of distilled water. This is a viscous solution which is included to maintain pH.

Erichrome Black T (EBT): A 0.2g of EBT was weighed and dissolved in a mixture of 50ml methanol (85%) and 2 g hydroxylamine hydrochloride. This indicator is for determining $Ca^{2+} + Mg^{2+}$.

0.02N EDTA Solution (Versenate): 3.723g of disodium ethylenediamine tetra-acetate dehydrate was diluted with distilled water to 1000ml and standardized against magnesium solution with EBT indicator to determine the presence of Ca^{2+}.

Calcium standard (0.02 N): A 1.0g of reagent grade calcium carbonate ($CaCO_3$) was dissolved in 1ml of concentration hydrogen chloride and diluted to 1000ml with distilled water.

Magnesium standard (0.02 N): A 2.465g of reagent grade magnesium sulfate heptahydrate was dissolved in 1000ml distilled water.

4.3.4.6 Determination of Calcium

Five millilitresof each of the sample solutions was transferred into 100ml Erlenmeyer flask. Ten millilitres of 10% potassium hydroxide solution was added, followed by 1 ml of 30% TEA. Three drops of 10% potassium cyanide and two drops of EBT indicator solution were put in the suspension. The mixture was agitated to ensure homogeneity and titrated with 0.02 N EDTA solution to change the colour from red to blue. The concentration was calculated, using formula developed by IITA (1979).

Calculation:

$$Ca^{2+}mg/kg^{-1} = \frac{0.02 \text{ x V x } 1000}{\text{Wt of the sample}}$$

Where:

W= weight in grammes of soil extracted

V= ml of 0.02 N EDTA used in the titration

0.02=concentration of EDTA used

4.3.4.7 Determination of Magnesium

Five millilitres of each sample solution was placed in a 100ml Erlenmeyer flask. Five millilitres of ammonium chloride-ammonium hydroxide buffer solution was added, followed by 1ml 30% TEA. Three drops of 10% potassium cyanide and a two drops of EBT indicator solution were added. The mixture was stired for 2min to ensure homogeneity and titrated with 0.02 N EDTA to change the solution from red to blue.The concentration of magnesium was calculated using formula developed by IITA (1979).

Calculation:

$$\text{Mg mg/kg}^{-1} = \frac{0.02 \times V \times 1000}{\text{Wt of the sample}}$$

Where:

W= weight in grammes of soil extracted

V= ml of 0.02 N EDTA used in the titration

0.02=concentration of EDTA used

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4.3.5Determination of Hydrogen Cyanide Content in Fresh and Dry Cassava Peels

The total cyanide content was determined in fresh and dry cassava peels, using Cooke (1978)method. Both dry and fresh cassava peels were separately cut into smaller piecesand thoroughly mixed separately. Sixty grammeswere weighed from each amendment,using electronic scale and placed in a glass beaker. Hundred and fifty millilitres of 0.1M orthophosphoric acid solution was added onto the chopped peels. The content in the beaker was stirredwith a glass rod for 2min and the suspension was filtered, using a cheesecloth.About 0.1ml of the suspension, 0.1ml of linimarase and 0.4ml of pH buffer 7 were incubated in shaking water bath at 30ºC for 15 min. At the end of the 15min period, 0.6ml of 0.2M sodium hydro-oxide was added to stop the reaction of linimarase. Chloramin T reagent (0.2 ml) was then added followed by

0.5ml of pyridine/pyrazolone reagent and left to stand for 90min for colour to develop. Different standards ranging from 0 to1.65µg of HCN were prepared and colour was developed. The absorbance of the different standards and samples were spectrophotometrically read at 620nm. The cyanide content of each sample was determined from the standard calibration curve on the photometer. The amount of cyanide in 100g sample was computed using the formula developed by Cooke(1978).

$$\text{ml/kg cyanide} = \frac{\mu g\ of\ cyanide\ x\ final\ volume\ (ml)}{Sample\ weight} \text{ x } 10$$

Where: µg/ml cyanide obtained from calibration curve

Final volume – volume of sample measured from filtered extract

Sample weight – weight of sample extracted

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4.3.6Preparation of the Aqueous Extracts of the Organic Amendments

The sweet orange and cassava peels were cut into small pieces of approximately 0.5cm width and 0.5cm length. Portions of the fresh peels of sweet orange and cassava were air-dried under shade in the plant house for two weeks to obtain the dry peels. For standardization, a sub-sample of 100g each of fresh and dry peels of sweet orange and cassava were oven-dried separately at 70°C for 72h and then weighed to determine the dry matter content (Loumedjinon *et al*., 2007). Fifty grammes of each type oforganic material (peels and rice husk)was separately soaked in 150ml of distilled water (1:3 w/v dilution) for 24h in a beaker at 28°C. The aqueous extract of each type of organic material was collected by sieving through cheesecloth. For the poultry manure, 50 g was placed in a cheesecloth and dipped into 150 ml of distilled water for 24 h. Twenty millilitres (20ml) of each aqueous extract was dispensed into separate 9cm diameter Petri dishes to determine their efficacy on egg hatching inhibition and juvenile mortality.

4.3.7Sterilization of Soil for Culturing of Root-knot Nematodes

Top soil was mixed with river sand at a ratio of 3:1. The resultant soil mixture was sieved with 2mm diameter sieve to remove debris and stones. The soil mixture was placed in a metal barrel and heated on fire from fuel wood at about 80°C to 90°Cfor 3h. After steam-sterilization, the soil was allowed to cool to room temperature before used forthe filling of the plastic pots (2 litres).

4.3.8Culturing and Extraction of Root-knot Nematode Juveniles and Eggs In order to obtain pure root-knot nematodes culture, egg masses were collected from infested-tomato roots from a farmer‟s field near KNUST. Plastic buckets (10 litres) were each filled with eight litres of the steam-sterilized soil mix and 21-day-old tomato seedlings nursed in sterilized top-soil were transplanted. The plants were inoculated with 2,000 root-knot nematode eggs, two weeks after transplanting and allowed to grow for two months in the plant house. Two months after inoculation, the plants were uprooted and roots were washed gently under tap water to remove soil. The root-knot nematode juveniles in the roots were extracted, using the modified Baermann tray method (Whitehead and Hemming, 1965).The roots were cut into pieces of about 0.5cm length with a pair of scissors and then placed in a plastic sieve lined with a two-ply tissue paper and placed in a plastic plate. Tap water was poured gently into the plastic plate in which the sieve was placed until the roots became moist. The set-up was left for 48h and the nematode suspension in the plates were then poured into separate beakers for counting. The root-knot nematode eggs were extracted, using modified Hussey and Barker method (1973). About 20g of the chopped roots were placed in a plastic bottle and 0.5% sodium hypochlorite (NaOCl) solution was added to cover the roots and then the bottle was covered. The content of the bottle was shaken vigorously for three min. The root and NaOCl mixture was collected in 200µm-pore mesh sieve over 500µm-pore mesh sieve and rinsed with tap water. The eggs were then washed into a beaker with a wash-bottle for counting.

4.3.9Assessment of Root-knot Nematode Juvenile Mortality

A total of 100 root-knot nematode juveniles were placed in each Petri dish containing the 20ml of the aqueous extract from each treatment. The number of dead root-knot nematodes was counted at 1, 2 and 3 days after inoculation. The immobile nematodes

were assumed dead and were then transferred into Petri dishes containing sterilized water. The Petri dishes were left on the laboratory bench at about28°C for another 24h. Nematodes were probed with a sharp-point needle under a stereo microscope and the ones that were straight in shape and remained immobile even after probing were considered dead (Amin, 1994).

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4.3.10Assessment of Root-knot Nematode Egg Hatch Inhibition *invitro*

One hundred (100) eggs were counted with the aid of a stereo microscope and placed in Petri dishes containing 20ml of each aqueous extract. The Petri dishes were placed on a laboratory bench at about 28° C for nine days. The number of eggs hatched was counted at 3, 6 and 9 days after inoculation.

Counting of the hatched juveniles from the eggs and dead nematodes was carried out, using a stereo microscope. Square lines were made at the underside of the Petri dish and all the juveniles and eggs in each square were counted, using the formula by Finney (1978).

a) Percent juvenile mortality = $\frac{Number\ of\ dead\ juveniles}{Total\ number\ of\ juveniles\ inoculated\ (100)}$ x 100

b) % inhibition of egg hatching

$$= \frac{Total\ number\ of\ eggs\ inoculated - No\ of\ eggs\ hatched}{Total\ number\ of\ egg\ inoculated\ (100)}\ x\ 100$$

4.3.11Data Collection

1. Number of dead juveniles: the number of dead juveniles were counted after inoculation and percent mortality was calculated for each treatment, using the formula by Finney (1978). Mortality was calculated as the percentage of dead nematodes out of the total number of juveniles inoculated.

2. Number of eggs hatched: the number of eggs hatched was counted after inoculation and percent eggs inhibition was calculated using the formula by Finney (1978).

4.3.12Data analysis

ANOVA was conducted, using GenStat statistical package, version 12.1. Where significant differences were found, Turkey‟s test was used to determine differences between means.

4.4Results

4.4.1 Root-knot Nematode Eggs Hatched at 3, 6 and 9 Days after Inoculation in Different Aqueous Extracts *invitro*

The effect of organic amendments (sweet orange and cassava peels) on root-knot nematode egg hatch inhibition at 3, 6 and 9 days after inoculation is presented in Table 4.1.There were significant effects ($P<0.05$) between the aqueous extract of sweet orange and cassava peels and observation times after inoculation on the egg hatching inhibition of root-knot nematodes. Significant differences ($P<0.05$) were observed between aqueous extract of fresh sweet orange peel and the rest of the treatments in all the observations, except FCP aqueous extract at three days after inoculation (Table 4.1). However, all the aqueous extracts of sweet orange and cassava peels had significantly higher ($P<0.05$) percentage egg hatch inhibition than sterilized water (Table 4.1). No significant differences ($P>0.05$) were observed between aqueous extract of FOP,FCP, DOP and DCP at three days after inoculation.

Table 4.1 Effect of different Aqueous Extracts on Eggs Hatchingat 3, 6 and 9 Days after Inoculation

Treatment	Percentage egg hatch inhibition		
	3 DAI*	**6 DAI**	**9 DAI**
Dry cassava peel (DCP)	85.1 b	72.6 c	70.1 c
Dry sweet orange peel (DOP)	89.6 ab	76.6 bc	74.8 bc
Fresh cassava peel (FCP)	91.0 a	81.0 b	79.1 b
Fresh sweet orange peel (FOP)	93.1 a	88.4 a	87.8 a
Poultry manure (PM)	76.6 c	32.2 d	32.2 d
Rice husk (RH)	55.2 d	12.2 e	3.4 e
Sterilized water (control)	54.2 d	10.5 e	1.9 e
LSD	**5.3**	**6.8**	**5.2**
CV (%)	**5.4**	**4.8**	**5.1**

Means followed by different letters in the same column are significantly different, according to Tukey test at $P<0.05$

*DAI = Days After Inoculation

There were significant differences (P<0.05) between poultry manure and rice husk and sterilized distilled water at all the observation dates. However, no significant difference (P>0.05) was observed between rice husk and sterilized distilled water in terms of egg hatching inhibition.

In terms of observation dates, the percentage egg hatching inhibition was observed to decline in all the treatments tested, but the rate of decline was higher in the poultry manure, rice husk and distilled water than the aqueous extracts of the sweet orange and cassava peels.The FOP had the lowest reduction in percentage egg inhibition, compared to the rest of the treatments over time (Table 4.1).

4.4.2 Mortality of Root-knot Nematode Juveniles in the different Aqueous Extracts *invitro*

Table 4.2 shows the effect of aqueous extracts of the organic amendments on the mortality of root-knot nematode juveniles at 1, 2 and 3 days after inoculation. The result of the percentage mortality followed a similar trend at all the observation periods. The aqueous extract of FOP had the highest nematode mortality in all the three periods observed (45.6, 86.6 and 94.8% of juveniles), followed by FCPaqueous extract (36.2, 71.0 and 86.6% of juveniles) and DOPaqueous extract (35.4, 70.6 and 86.4% of juveniles), respectively. No mortality was observed in the rice husk aqueous extract and sterilized water throughout the period of observation(Table 4.2).

Table 4.2Mortality of Root-knot Nematode Juveniles in different Aqueous Extracts *invitro*

Treatment	Percent mortality		
	1 DAI*	**2DAI**	**3 DAI**
Dry cassava peel (DCP)	22.0 c	57.0 c	62.6 c
Dry sweet orange peel (DOP)	35.4 b	70.6 b	86.4 b
Fresh cassava peel (FCP)	36.2 b	71.0 b	86.6 b
Fresh sweet orange peel (FOP)	45.6 a	86.6 a	94.8 a
Poultry manure (PM)	3.6 d	11.6 d	24.0 d
Rice husk (RH)	0.0 e	0.0 e	0.0 e
Sterilized water (control)	0.0 e	0.0 e	0.0 e
LSD (P<0.05)	**1.1**	**0.5**	**0.3**
CV (%)	**6.2**	**6.5**	**5.9**

Means followed by differentletters in the same column are significantly different, according to Tukey test at*P*<0.05

*DAI = Days After Inoculation

Significant differences (P<0.05) were observed between aqueous extract of FOP and the rest of the treatments applied in the observation periods (12, 48 and 72h) (Table 4.2). Similarly, FCP and DOP aqueous extracts were significantly different (P<0.05) from DCP aqueous extract, rice husk aqueous extract and sterilized water. However,

FCP and DOP aqueous extracts were not significantly different (P>0.05) from each other (Table 4.2).

For the effect of the aqueous extract of sweet orange and cassava peels on mortality of root-knot nematode juveniles over time, the rate of mortality significantly increased (P<0.05) with time after inoculation from 12 to 72h (Table 4.2). The rate of mortality in the aqueous extract of FOP was significantly higher (P<0.05) than the rest of the test treatments in all the observations. There was no significant difference (P>0.05) between aqueous extract of rice husk and sterilized water in all the observations. Comparing the fresh and dry peels, the percentage mortality rate was observed to be significantly higher (P<0.05) in the aqueous extracts of fresh peels than the dry peels in all the observation periods (Table 4.2).

4.5Experiment 2: Evaluation of Aqueous Extracts of fSweet Orange and Cassava Peel or Their Nematicidal Potentials on Root-Knot Nematodes of Tomato in the Plant House (In vivo)

4.5.1 Materials and Methods

4.5.1.1 Condition in the Plant House

The experiment was carried out in the plant house, Faculty of Agriculture, KNUST, from 8th May to 8th July, 2014. The mean daily temperature and relative humidity in the plant house were: 24to 32°C (temperature) and 60 to 89% (relative humidity). The soil used consisted of 84.1% sand, 6.3% silt and 9.6% clay with pH of 7.89.

4.5.1.2 Experimental Design

The experimental design was Completely Randomized Design (CRD) replicated five times. A rate of 50g dry matter/plant was used for the peels and rice husk (Loumedjinon *etal*.; 2007). The poultry manure and carbofuran were applied at rates of 100g and 0.5g/plant, respectively (Amulu and Adekunle, 2015).

The following treatments were used:

1. 50g/plant of fresh sweet orange peel
2. 50g/plant of dry orange peel
3. 50g/plant of fresh cassava peel
4. 50g/plant dry cassava peel
5. 50g/plant of rice husk
6. 100g/plant of poultry manure
7. Control- sterilized soil (no application)
8. 0.5g/plant of carbofuran

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4.5.1.3 The Tomato Variety used and its Characteristic

The seed of locally preferred tomato variety "power" was obtained from the CSIRCRI. Power has growth duration of 90 days and the variety is found to be susceptible to root-knot nematodes (Kwara *etal*., 2014). The variety has an average yield of 17 Mt/ha in farmers‟ fields where inputs are adequately applied (Robinson and Kolavalli, 2010). Ninety-five percent (95%) germination was obtained when germination test of the seeds was conducted.

4.5.1.4Type and Sterilization of Soil

The type soil used was steam-sterilized as described at section 4.3.7.

4.5.1.5 Productionof Tomato Seedlings used for the Plant House Experiment The tomato seeds were nursedin seed boxes filled with steam-sterilized sandy-loam soil. The seeds were sown in rows at a depth of about 2cm and 15cm apart. Soil moisture was maintained by applying 50ml of water every other-day for 21 days. The seedlings were then used for the study.

4.5.1.6 Application of the Treatments in Steam-sterilized Soil in Pots

The treatments listed at section 4.5.1.2 were uniformly applied at four weeks before transplanting the tomato seedlings. Each organic amendment was thoroughly mixed with 1.8 L of steam-sterilized soil and filled in 2 L sizedpots. The soil used was topsoil-river sand mixture at a ratio of 3:1. Tap water was sprinkled on the soil every three days for four weeks to facilitate decomposition of the amendments.

4.5.1.7 Application of Carbofuran

The Carbodan 3% G carbofuran (*2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate*)(Shenzhen Baocheng Chemical Industry, China) was applied four weeks before transplanting at the rate of 0.5g per pot (Amulu and Adekunle, 2015). The chemical was thoroughly mixed with 1.8 L of steam-sterilized soil in 2 L sized pots. Water was sprinkled on the soil every three days for four weeks to allow the chemical to dissolve.

4.5.1.8 Transplanting of Tomato Seedlings

Twenty-one day-old tomato seedlings were transplanted into 2 L sized pots containing 1.8 L of steam-sterilized topsoil-river sand mixture. One tomato seedling was transplanted into each pot. The topsoil-river sand mixture was moistened before transplanting the seedlings. Uniform seedlings, in terms of height, were selected and transplanted. One hundred millilitres (100ml) of tap water was applied to each plant in the pot every two days. A plate was placed under each pot and drained water collected in it was poured back into the pot after watering.

.1.9 Extraction and Determination of Number of Root-knot Nematode Eggs for Inoculation

The root-knot nematode eggs were obtained from roots of nematode-infested tomato plants cultured in the plant house. The eggs were extracted using the modified Hussey and Barker, (1973)method. One millilitre (1ml) of the egg suspension was collected with a syringe after thorough mixing by blowing air into the suspension with a pipette. The suspension was poured on the counting tray and mounted on the inverted compound microscope(Leica, Leica Microsystems Company Ltd., Germany) to determine the number of eggs.

4.5.1.10 Inoculation of Root-knot Nematode Eggs in the Soil at the Plant House A total of 1500 root-knot nematode eggs were inoculated into each pot containing steam-sterilized soil at two weeks after transplanting. The eggs were inoculated into the soil at three different points at a distance of 2cm from the base of the plant. The eggs were placed in the soil at a depth of 5cm using a pipette.

4.5.1.11 Harvesting of Plants for Extraction of Root-knot Nematode Eggs and Juveniles

Theplantswere harvested at eight weeks after transplanting. Each plant was uprootedwith soil around the roots and properly labeled for nematode extraction at the laboratory.

4.5.1.12 Extraction of Root-knot Nematodes Eggs from Rootsof Tomato after Harvest

The harvested root from each pot was washed with tap water to remove soil and debris. They were then dabbed dry with tissue paper. The roots were chopped with a pair of scissors. The eggs were extracted using the modified Hussey and Barker method (1973). Five grams (5g) of chopped roots were collected and placed in a plastic bottle containing 0.5% sodium hypochlorite (NaOCl) solution. The bottle was covered and

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agitated for three min. The content was poured into 200µm-pore mesh sieve over 500µm-pore mesh sieve and rinsed with tap water. The root-knot nematode eggs collected in the 500µm-pore mesh sieve were washed into a beaker using a wash-bottle.

4.5.1.13 Extraction of Root-knot Nematode Juvenilesfrom the Soiland Roots of Tomato

The root-knot nematodes in the soil and roots were extracted using the modified Baermann tray method (Whitehead and Hemming, 1965). One hundred grammes (100g) of the soil and five grammes of roots were separately placed in a plastic sieve lined with a two-ply tissue paper spread in a plastic plate. Tap water was poured gently into the plastic plate containing the sieve until the soil/roots became moist. The set-up was left for 48h and the plates were then poured separately into beakers and left overnight for the juveniles to settle.

4.5.1.14 Counting of Root-knot Nematode Eggs and Juveniles

The nematode juveniles-water suspension in the beaker was adjusted to 50ml by adding(depending on the initial volume of the suspension)tap water to each suspension. The suspension was homogenized by blowing air into it using a pipette. One millilitre (1ml)aliquot of the suspension was collected using a pipette and poured into a counting trayunder an inverted compound microscope(Leica, Leica Microsystems Company Ltd., Germany). The nematodes and eggs were counted three times for each sample. The means were calculated and extrapolated to number of juveniles or eggs/100ml of soil and per 5g of root.

.1.15 Nutrient Analyses of the Organic Amendments

Samples were collected from each organic amendment and taken to the Soil Science laboratory of the Department of Crop and Soil Sciences, KNUST, for analysis. The samples were analyzed as described for experiment 1.

4.5.1.16 Data Collection

Data collected from the experiment were as follows:

1. **Nutrient content of organic amendments:**Nutrient content of the organic amendments was determined as described for experiment 1.
2. **Plant height at harvest:**At harvest, plant heights were measured from the base of the stem to the tip of the tallest branch, using a measuring tape
3. **Fresh shoot weight:**The shoot of the plant was cut from the base of the stem (collar) and weighed. The fresh shoot was weighed immediately after harvest using electronic scale (Maestro CKX53, Myweight Co. Ltd., USA).
4. **Fresh root weight:**The roots were cut from the base of the stem (collar) and weighed, using electronic scale (Maestro CKX53, Myweight Co. Ltd., USA). Each plant root was weighed separately to determine the fresh rootweight.
5. **Root length:**The length of the root was measured from the base of the stem to the tip of the longest root, using a meter rule.
6. **Gall index:**The roots were placed in a transparent glass bottle containing tap water andallowedto spread.The galls were rated by comparing with the diagrammatic root-knot scoring chart provided by Bridge and Page (1980) (Appendix 55).
7. **Number of nematodes and eggs from the soil and roots:**The number of root-knot nematode juveniles and eggs from the roots and soil was counted after harvest of tomato plants. One millilitre of the nematode and egg suspensions were separately placed on a counting tray under inverted compound microscope and each was counted three times. The mean was calculated to determine the number of root-knot nematodes and eggs in the soil and root of tomato.

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8. **Reproduction factor:** The reproduction factor was calculated by dividing the final population (Pf) by the initial population (Pi) (Pf/Pi).

4.5.2 Results

4.5.3 Pot Evaluation of Aqueous Extracts of the Organic Materials for Their Nematicidal Potentials on Root-knot Nematodes

4.5.3.1 Concentration of the Chemical Nutrients in the Organic Materials The poultry manure generally, had highest values in all the parameters measured except organic carbon and potassium(Table 4.3). However, values for the mineral elements except pH were found to be higher in the sweet orange peel than cassava peels. For the hydrogen cyanide content in fresh cassava peel and dry cassava peel, the highest concentration was observed in the fresh cassava peels compared with the dry peel. The concentration of hydrogen cyanide was reduced by about 50% when the fresh cassava peel was dried under shade for two weeks (Table 4.3).

Table 4.3 Chemical Composition of the Organic Materials Used for Root-knot Nematodes Management

Organic amendments	% Organic Carbon	% Total Nitrogen	K mg/kg	Na Mg/ Kg	Ca mg/kg	Mg mg/kg	Avail. P mgkg	pH	Total Hydrogen Cyanide
Poultry manure	50.67	2.74	2.45	0.34	3.01	1.88	0.94	8.33	
Orange peel	63.04	1.16	3.76	0.27	0.62	0.44	0.13	5.75	-
Cassava peel	56.66	0.76	2.63	0.19	0.36	0.29	0.04	6.75	150.0 (330.0*)

*Value for cyanide in fresh cassava peel

.3.2 Physical and Chemical Analysis of the Soil Samples used in the Pot Experiment at the Plant House

Table 4.4 presents the results of the soil analysis carried out on the physical and chemical properties. The soil type used for the pot experiment at the plant house was loamy sand. The concentration of chemical nutrients found were: Calcium (10.4mg/kg),

Total nitrogen (0.06%), Potassium (0.10mg/kg), Sodium (0.09mg/kg) and Magnesium (0.76mg/kg) (Table 4.4).

Table 4.4 Physical and Chemical Analysis of the Soil Sample used in the Plant House

Soil	Results
Physical properties	
Silt (%)	6.28
Clay (%)	9.60
Sand (%)	84.12
Chemical properties	
Total N (%)	0.06
Sodium (mg/kg)	0.09
Potassium (mgl/kg)	0.10
Magnesium (mg/kg)	0.76
Organic carbon (%)	1.35
0rganic matter (%)	2.33
pH	7.89
Calcium (mg/kg)	10.40
Available P. Acidity (mg/kg)	10.47

Source: Soil Research

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4.5.3.3 Effect of Organic Amendments on Plant Growth Factors at Eight Weeks after Transplanting

The effect of organic amendments on plant growth factors at eight weeks after transplanting is shown in Table 4.5. Poultry manure was observed to significantly increase ($P<0.05$) shoot length (58.0 cm) of tomato plant than the rest of the treatments tested. The lowest plant shoot length was obtained where rice husk was applied (37.0 cm). Significant differences ($P<0.05$) were observed between poultry manure and all the other treatments (Table 4.5). Similarly, FCP and FOP were significantly different ($P<0.05$) from carbofuran, rice husk and no application

(control). However, no significant differences ($P>0.05$) were observed between FOP, FCP, DOP and DCP. Also, rice husk and control were not significantly different ($P>0.05$) from each other for shoot length of tomato (Table 4.5).

4.5

The application of poultry manure significantly increased (P<0.05) root length of tomato in the plant house more than all the other treatments applied (Table 4.5). There were no significant differences (P>0.05) between carbofuran, FOP, FCP, DOP and DCP, but they were significantly different (P<0.05) from no application (control) (Table 4.5).

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Table 4.5 Effect of Organic Amendments on Plant Growth Factors at Eight Weeks after Transplanting in the Plant House

Treatments	Shoot length (cm)	Root Length (cm)/plant	Fresh shoot weight (g)/plant	Fresh root weight (g)/plant
Fresh sweet orange peel (FOP)	49.0 b	8.1 b	19.6 bc	4.3 cd
Fresh cassava Peel (FCP)	49.6 b	8.3 b	19.9 bc	4.3 cd
Dry sweet orange peel (DOP)	46.7 bc	7.3 b	19.4 bc	4.0 d
Dry cassava Peel (DCP)	45.7 bc	7.2 b	18.8 cd	4.7 abc
Poultry Manure	58.0 a	9.8 a	20.3 a	4.7 bc
Rice Husk	37.0 d	7.2 b	16.1 e	5.0 ab
Carbofuran	42.7 c	8.1b	17.9 d	4.5 bcd
Control	37.4 d	5.9 c	15.8 e	5.3 a
LSD (p<0.05)	**4.9**	**1.1**	**1.3**	**0.6**
CV (%)	**8.4**	**11.8**	**5.4**	**10.7**

Means followed by different letters in the same column are significantly different, according to Tukey"stest at *P*<0.05

NB Carbofuran was used as positive control to the organic amendments tested because there was no other chemical nematicide available at the moment.

The highest fresh shoot weight/plant was recorded from poultry manure treated pots (20.34g), followed by the application of FCP (19.9g) (Table 4.5). The lowest fresh shoot weight/plant was obtained from the no application (control) (15.8g) (Table 4.5). There were significant differences (P<0.05) between poultry manure and the rest of

the treatments. Also, FOP and FCP were significantly different (P<0.05) from carbofuran, rice husk and no application (control). No significant differences (P>0.05) were observed between carbofuran and dry cassava peel (Table 4.5).

The fresh root weight/plant was significantly increased (P<0.05) in the no application (control) than the rest of the treatments except the application of rice husk (Table 4.5). Significant differences (P<0.05) were observed between no application (control) and FOP, poultry manure, DOP, FCP and carbofuran. There were no significant differences (P>0.05) observed between poultry manure, FOP, FCP and DCP for the fresh root weight (Table 4.5).

4.5.3.4 Root Galling and Number of Juveniles Extracted from the Root and Soil under Different Treatments in the Plant House

The gall indexobserved on the roots of tomato in the plant house was significantly lower (P<0.05) in the application of FOP(85.4%) and carbofuran (87.8%) than all the other treatments(Table 4.6). Significant differences (P<0.05) occurred between carbofuran and all the other treatments except FOP. Also, FCP and DOP were significantly different (P<0.05) from DCP, poultry manure, rice husk and no application (Plate 4.1). No significant difference (P>0.05) was observed between FCPand DOP. Similarly, rice husk and control were not significantly different (P>0.05) from each other (Table 4.6).

Plate 4.1 Severity of Galls under Fresh Orange Peels (A) Carbofuran (B) and no

Application (C) at the Plant House

The percentage of eggs laid by root-knot nematodes on tomato roots were significantly reduced ($P<0.05$) in the application of FOP and carbofuran than the rest of the treatments tested (Table 4.6).

The largest number ofroot-knot nematode eggs in the roots was obtained inthe no application(2856 eggs/5 g root) and smallest number of eggs was observed in the application of carbofuran (463 eggs/5 g root) (Table 4.6).Significant differences ($P<0.05$) were recorded between carbofuran and the rest of the treatments except application of FOP. Similarly, FOP was found to be significantly different ($P<0.05$) from poultry manure, DOP, DCP, rice husk and no application. (Table 4.6).

Carbofuran significantly reduced ($P<0.05$) the number of root-knot nematodes in the roots than all the other treatments tested, followed by FOP (Table 4.6). The highest number of root-knot nematodes extracted was observed in the no application (Table 4.6). Significant differences ($P<0.05$) were observed between carbofuran and the rest of the treatments (Table 4.6). Also, FOP was significantly different ($P<0.05$) from DOP, FCP, poultry manure, DCP, rice husk and no application (control) (Table 4.6).

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Table 4.6 Gall Index, Number of Eggs, Number of Juveniles in the Root and Soil and Reproduction Factor of Root-knot Nematodes as Affected by Organic Amendments in the Plant House

Treatments	[x] Root-knot nematode gall index	% reduction of root galling	[z] Root-knot nematode eggs/5g root	[z] Root-knot nematode juveniles/5g root	[z] Root-knot nematodes 100 ml soil	[y]RF
Fresh sweet orangepeels (FOP)	1.2 f	85.4	680 d	167 f	528 e	0.5
Fresh cassava peels (FCP)	2.0 e	75.6	1288 c	268 e	648 d	0.6
Dry sweet orange peel (DOP)	2.0 e	75.6	1302 c	286 e	674 cd	0.6
Dry cassava peels (DCP)	3.2 d	61.0	1473 c	564 d	749 c	0.9
Poultry Manure	6.4 c	22.0	2323 b	735 c	1239 b	1.3
Rice Husk	7.6 a	7.3	2843 a	963 b	1999 a	2.0
Carbofuran	1.0 f	87.8	463 d	117 f	416 f	0.4
No application (Control)	8.2 a	-	2856 a	1029 a	2066 a	2.1
LSD (p<0.05)	**0.6**	**-**	**187.9**	**19.6**	**27.8**	
CV (%)	**12.3**	**-**	**5.3**	**2.8**	**3.0**	

Means followed by different letters in the same column are significantly different, according to Tukey"s test at *P*<0.05

[z] All data were back-transformed after transformation using $\sqrt{}$ [y]Reproduction factor = Final population (Pf)/Initial population (Pi), where Pi = 1500 [x]Galling on scale 0-10, where 0 = no galls and 10 = root system completely galled (Bridge and Page, 1980).

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The application of carbofuran significantly reduced (P<0.05) root-knot nematode population in the soil more than the rest of the test treatments(Table 4.6). The largest number of root-knot nematodes extracted from the soil was observed in the no application (Table 4.6).Carbofuran was significantly different (P<0.05) from all the other treatments in terms of reducing the population of root-knot nematodes in the soil (Table 4.6). Similarly, significant differences (P<0.05) were observed between FOP and FCP, DOP, DCP, poultry manure, rice husk and no application (control). Also, significant differences (P<0.05) occurred between poultry manure and rice husk and no application (control). No significant difference (P>0.05) was observed between the application of DOP and FCP (Table 4.6).

The reproduction factor(RF) of root-knot nematodes under the various treatments varied from 0.4 to 2.1 (Table 4.6). The highest reproduction factor was recorded in no application (control), followed by rice husk and poultry manure. The lowest reproduction factor was observed where carbofuran and FOPwere applied (Table 4.6).

EXPERIMENT THREE

4.6Experiment 3: Effect of Sweet Orange and Cassava Peels and their Combinations on the Management of Root-knot Nematodes in the Field

4.6.1 Material and Methods

4.6.1.1 Description of the Experimental Location

The experimental site was at the research field of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana located on longitude 01° 33´W and latitude 06° 41´N. The area has bimodal rainfall pattern, with highest rainfall in May/June and October for the major and minor seasons, respectively. Mean annual rainfall is between 1100 and 1800mm. The site was previously grown to tomato, pepper and eggplant two months prior to the experiment.

Before conductingthe experiment, weed samples were collected from the field and identified. The most predominant weeds were *Cyperusrotundus* (Linn.) and *Panicummaximum* (Jacq.).

4.6.1.2 Experimental Design

The experiment was arranged in Randomized Complete Block Design (RCBD) with four replications. Plot size was 2m x 4m with walk-way of 1m between plots and blocks. The experiment was repeated for three seasons at the same location; the first experiment was carried out during the major season (June to September, 2014), the second during the minor season (October, 2014 to December, 2014) and the third experiment was carried out in the major season of 2015 (July to September). The amendments were not applied in the third season to help determine the residual effects of the various treatments on root-knot nematodes. The experiment was carried out under natural conditions without any nematode inoculation.

The poultry manure and rice husk were excluded in this experiment because of their poor performance in terms of nematode management in the plant house. The experiment consisted of the following treatments:

1. 50g/plant of fresh sweet orange peel (FOP)
2. 50g/plant of dry sweet orange peel (DCP)
3. 50g/plant of fresh cassava peel (FCP)
4. 50g/plant of dry cassava peel (DCP)
5. Zero application (control)
6. 25g/plant of FCP + 25g/plant of FOP
7. 25g/plant of FOP + 25g/plant of FCP
8. 25g/plant of DOP+ 25g/plant of DCP
9. 25g/plant of DCP+ 25g/plant of DOP
10. NPK (15:15:15) fertilizer + Ammonium sulphate ($(NH_4)_2\ SO_4$)

4.6

11. Carbofuran (synthetic nematicide)

.1.3 LandPreparation for Transplanting of Tomato Seedlings

Weeds at the experimental site were cleared by using cutlass, and ridges were raised by using a hoe to a height of about 20cm. The ridges were spaced at 90cm apart and small bunds (tie-ridges) were constructed between them to help reduce erosion in the furrows.

4.6.1.4 Preparation and Application of the Organic Amendments and Carbofuran to the Soil

Both fresh and dry peels of cassava and sweet orange were used. The weight of fresh and dry peels was determined based on dry matter content as described at Section 4.3.6. Each type of peel was applied at the rate of 50g dry matter/plant (Loumedjinon *etal*., 2007). The single dose treatments were applied four weeks before seedlings were transplanted. The first part (25g) of the combined treatments was applied four weeks before transplanting while the remaining (25g) at three weeks after transplanting. The amendments were placed around the plant in the form of a ring at 5cm radius from the base of each plant and thoroughly incorporated into the soil with a hand fork to a depth of 10cm and covered with soil to facilitate decomposition and distribution of the constituents into the soil. Carbodan 3% G (Carbofuran)was applied at four weeks before transplanting at the rate of 0.5g per plant (Amulu and Adekunle, 2015) and incorporated as the organic amendments.

4.6.1.5 Transplanting of Seedlings and Application of Fertilizers

The seedlings were grown in boxes filled with steam-sterilized top-soil. The seeds were sown in rows at a depth of 2cm and 15cm apart. Four-week-old tomato (var.

Power) seedlings were transplanted onto the ridges at a spacing of 50cm intra-row and 90cm inter-row. Seven plants were transplanted in each row with a total of 14 plants in each plot. Vigorous and equal height seedlings were used to maintain uniformity.

NPK (15: 15:15) (Zouping Runzi Chemical Industry Co., Ltd., China)was applied at a rate of 250kg/ha (200 g/plot) at four weeks after transplanting. In addition, ammonium sulphate ($(NH_4)_2SO_4$) was applied at a rate of 125kg/ha (100 g/plot) (Yeboah *et al*., 2014) at six weeks after transplanting. Both fertilizers (NPK and $(NH_4)_2SO_4$) were applied in rings at 5cm radius and 10cm deep from the base of each plant and covered with soil.

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4.6.1.6 Application of Fungicides and Insecticides

Merpan 50WP (Captan 500g/kg) and Folpan 50 WP (Folpet 500g/kg) fungicides made by Makhteshim Chemical Works Ltd, Israel were applied at the rate of 1.2kg/ha every two weeks. The insecticide Acceta Star (16g Acetamiprid/L and 30 g Bifenthrin/L) was applied every week at the rate of 1 L per hectare, but was later found to be infective against whiteflies, thus the insecticide was changed for Golan. Golan (200 g of Acetamiprid/L) was also applied at the rate of 0.5 L/ha every two weeks to control whiteflies. All pesticides were thoroughly mixed with water in a knapsack sprayer and applied. Both chemicals were manufactured by Makhteshim Chemical Works Ltd., Israel.

4.6.1.7 Cultural Practices

For each experiment, weeding was done three times, using a hoe.At six weeks after transplanting, the plants were staked, using sticks and rope to prevent fruits from lodging on the ground. Supplementary irrigation was carried out when the plants show water deficiency. Fifteen litres of tap-water was applied in each plot daily using a plastic watering container.

.1.8 Extraction of Root-knot Nematode Eggs and Juveniles from Roots and Soil

4.6

The tomato plant roots were uprooted at three months after harvest and taken to the Nematology laboratory for extraction of root-knot nematode eggs and juveniles. The roots were washed with running tap water and dabbed dry with tissue paper. Ten grammes of the chopped roots from each sample was used to extract root-knot nematode eggs and juveniles as described at section 4.5.1.12. During the root sampling, a ball of soil was collected together with the root. Each soil sample was separated from the roots and thoroughly mixed by hand and air dried on benches in the laboratory. One hundred millilitres (100ml) of soil from each sample was measured, using electronic scale (Maestro CKX53, Myweight Co. Ltd., USA). The root-knot nematode juveniles were extracted from the soil, using Baermann tray method (Whitehead and Hemming, 1965). The modified Hussey and Barker, (1973) method was used to extract the eggs from the roots.

4.6.1.9 Morphological Identification of Nematodes

Nematodes preserved in formalin and glycerol (Coyne *etal*., 2007) were used for identification. During identification, 1ml aliqout of the nematode suspension in the tube was collected with a syringe and then placed into the counting tray and then placed under the inverted compound microscope (Leica,Leica Microsystems Company Ltd,Germany). The images of the nematodes were recorded at magnifications 25x and 40x and were later used for identification using manuals (Eisenback *etal*., 1981). The following features were considered during observation to determine a particular genus: shape of the nematode at death, shape of the head, lip region, stylet shape, median bulb, position of the overlap, vulva position, spicule position, presence and absence of bursa and shape of the tail.

4.6

.2 Data Collection

Data were taken from soil and plant roots. Seven out of 14 plants in each plot were selected at random from each plot and permanently tagged for data collection.

4.6.2.1 InitialPopulation of Root-knot Nematodes

Before application of treatments, five core-soil samples were systematically collected from each plot at a depth of 0–15cm using a hand trowel. The samples were taken to the Nematology laboratory for extraction of root-knot nematodes.

4.6.2.2 PlantHeight at Harvest

Plant height (cm) was measured at harvest using a measuring tape. Plants were measured from the ground level up to the tip of the tallest branch. This was carriedout on the seven permanently tagged plants in each plot. The mean height was then calculated.

4.6.2.3 Number of Fruits and Yield (kg/ha)

The tomato fruits harvested from each of the seven plants were counted and weighed to determine the yield at maturity. The fruits were harvested when they were ripe (red colour). The number of fruits and yield data collected at different intervals were summed up to have the final data for each treatment.

4.6.2.4 Weights of Shoot and Roots at Harvest

At harvest, seven plants were randomly collected by uprooting from the net-plot (seven plants). The fresh shoot and roots were weighed separately to determine their weights.

.2.5 Number of Eggs and Nematodes in Roots and Soil at Harvest

Soil and root samples were collected at harvest for extraction of root-knot nematodes. A total of three plants were systematically uprooted from each plot. Each sample was

4.6

labelled and placed in plastic container and sent to the laboratory for extraction and counting. The counting of the eggs and nematodes were carried out using inverted compound microscope.

4.6.2.6 Determination of Gall Index of Root-knot Nematodes on Tomato Roots The roots were placed in a transparent glass bottle containing tap water. The roots were spread and galls were rated by comparing with diagram provided by Bridge and Page (1980) (Appendix 55).

4.6.2.7 Determination of Fungal Antagonists in the Soil

Before and after application of the treatments, five core-soil samples were collected at a depth of 0 -15cm and mixed to form a composite for each plot. The soil samples were dried without sunshine in the laboratory for 48h before isolation. Fungi were isolated by serial dilution at 10^{-6} and number of colonies for each fungus was counted and identified. Potato Dextrose Agar (PDA) was used to isolate the fungi and a compound microscope (Leica,Leica Microsystems company Ltd.,Germany), specimen and reference manuals (Watanabe, 2000) were used for identification. Parts of the same samples were used to extract and determine the population of free-living nematodes. The free-living nematodes were extracted from the soil, using Baermann tray method (Whitehead and Hemming, 1965).

4.6.2.8 Soil Chemical Analysis

During the experiments, soil samples were taken before transplanting and at harvest to determine the nutrient status of the soil. The samples were collected diagonally fromeach plot at a depth of 0-20cm, using soil auger. The samples were mixed together to have composite sample for each treatment. The samples were taken to the soil laboratory, Faculty of Agriculture, for analysis. However, before analysis, the samples

were dried under shade for three days and pounded, using mortar and pestle to break the aggregates into individual particles. These were later sieved with 2mm diameter sieve to remove stones and debris. The data collected were as follows:

4.6.2.8.1 Determination of Soil pH

Soil pH was determined by measuring in a 1:1 soil-water ratio using a glass electrode (H19017 Microprocessor) pH meter. Ten grams (10g) soil sample was placed in a 50ml beaker and 20ml of distilled water added. The suspension was stirred thoroughly for 10 seconds, using a glass rod and later stirred again for 30 min. Before taking the readings, the pH meter was calibrated with buffer solutions of pH 7.0 and 4.0. The pH was read by inserting the electrode into the upper part of the soil solution and the pH value recorded. The test for pH was repeated three times for each sample and the mean was calculated.

4.6.2.8.2 Determination of Soil Organic Carbon

Soil organic carbon was determined by the modified Walkley-Black method as described by Nelson and Sommers (1982). One gramme of soil sample was weighed and placed in a 500ml conical flask. In addition, a reference sample and a blank were included. Ten millilitres (10ml) of 0.16667M potassium dichromate ($K_2Cr_2O_7$) solution was added to 20ml of concentrated H_2SO_4 containing Ag_2SO_4. The suspension was mixed thoroughly and the reaction was allowed to complete for 30 min. Distilled water (250ml) and 10ml concentrated orthophosphoric acid were added

and allowed to cool. A diphenylamine indicator (1ml) was added to the mixture which was then titrated with 1.0Mferrous sulphate solution.

Calculation:

The organic carbon content of the soil was calculatedfollowing(Nelson and Sommers, 1982) procedure.

$$\% \text{ Organic carbon (OC)} = \frac{M \times 0.39 \times mcf \times (V^1 - V^2)}{w}$$

where:

M = molarity of ferrous sulphate solution

V_1 = ml of ferrous sulphate solution required for blank

V_2 = ml of ferrous sulphate solution required for sample

w = weight of air-dry sample in grams

$$mcf = \text{moisture correcting factor} = \frac{100}{100 - \text{moisture}} \%$$

0.39 = 3 x 0.001 x 100 % x 1.3 (3 = equivalent mass of carbon)

1.3 = a compensation factor for the incomplete combustion of the organic carbon.

The percent organic matter was calculated after the determination of organic carbon according to (Walkley and Black, 1934).

% Organic matter (OM) = % OC ×1.724

4.6.2.8.3 Determination of Available Phosphorus

Soil available phosphorus was determined, using the Bray P_1 method (Olsen and Sommers, 1982). About 2.0g of soil sample was weighed into a 50ml shaking bottle and 20ml of Bray-1 extracting solution (0.03 *N* NH_4F + 0.025 *N* HCl) was added.

The sample was shaken for one minute and then filtered through No. 42 Whatman filter paper. Ten millilitres (10ml) of the filtrate was pipetted into a 25ml volumetric flask and 1ml each of molybdate reagent and reducing agent added for colour development. The absorbance was measured at 660nm wave-length on a spectrophotometer. The concentration of P was calculated as (Olsen and Sommers,
1982).

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Calculation:

$$P\ (mg\ kg^{-1}) = \frac{(a - b) \times 35 \times 15 \times mcf}{w}$$

where:

a = mg/l P in sample extract b = mg/l P in blank w = sample weight in gram mcf = moisture correcting factor

35 = volume of extracting solution

4.6.2.8.4Determination of Soil Total Nitrogen

One grammme (1g) of soil sample was weighed and placed in a Kjeldahl flask. A 0.7g of copper sulphate, 1.5g of K_2SO_4 and 30ml of H_2SO_4 was added (Bremmer and Mulvaney, 1982) to the soil.The set up was heated gently until frothing ceased. Thefinal volume (15ml) of sample solution was then boiled briskly until the solution was clear and digested for 30 min. The flask was removed from the heater and cooled, 50ml of water was added and was then transferred into a distilling flask. A 20 – 25ml of standard acid (0.1MHCl) was placed in the receiving conical flask to get an excess of at least 5ml of the acid. Three drops of methyl red indicator was added and enough water was added to cover the end of the condenser outlet tubes. Tap water was run through the condenser before 30ml of 35% NaOH in the distilling flask was added. The content was heated to distil the ammonia for about 30 –40 min.The receiving flask was removed and the outlet tube was rinsed into the receiving flask with a small amount of distilled water. The excess acid was titrated in the distillate with 0.1MNaOH. The blank was determined on reagents by using the same quantity of standard acid in a receiving conical flask.

The total nitrogen was calculated using a formula developed by FAO (2008).

$$\text{Percent N} = \frac{1.401(V_1M_I - V_2M_2) - (V_3M_1 - V_4M_2) \times df}{W}$$

where: V_1= millilitres of standard acid put in receiving flask for samples

V_2= millilitres of standard NaOH used in titration

V_3= millilitres of standard acid put in receiving flask for blank

V_4= millilitres of standard NaOH used in titrating blank

M_1= molarity of standard acid

M_2= molarity of standard NaOH

W= weight of sample taken (1g) df= dilution factor of sample

4.6.3 Data analyses

The data collected from the experiments were subjected to ANOVA, using GenStat 12th edition software. The means were separated, using Tukey‟s test at $P<0.05$. Test for normal distribution was carried out and nematode and egg counts were square root transformed $\sqrt{(x+1)}$, where x is the mean count before statistical analysis and then back-transformed.

4.6.4 Results

Before the application of the organic amendments, the soil was found to be loamy sand Dystric Cambisol with a composition of 87.3% sand, 7.0% silt and 5.7% clay in the topsoil (0-15cm) (Table 4.7). The pH, percentage organic carbon, organic matter and total nitrogen were 7.4, 1.2, 1.35 and 0.10, respectively (Table 4.7).

Table 4.7Physical and Chemical Analysis of the Soil Sample taken before the Application of Amendments at the Field

Soil	Results
Physical properties	
Clay (%)	5.70
Silt (%)	7.00
Sand (%)	87.30
Chemical properties	
Total N (%)	0.10
Organic carbon (%)	1.21
Potassium (mgl/kg)	0.25
0rganic matter (%)	1.35
pH	7.40
Available P. Acidity (mg/kg)	48.47

Source: Soil research

4.6.4.1 Effect of Sweet Orange and Cassava Peels and their Combinations on Plant Growth Factors and Root-knot Nematodes in the Field

The number of plant-parasitic nematodes extracted from the soil before the application of treatments is presented in Table 4.8. The number of the nematodes varied from five to 686 nematodes/100ml soil. The highest percentage (41.6%) of nematodes at the study site was*Meloidogyne* spp., followed by *Helicotylenchus* spp. and *Pratylenchus* spp. with 23.6% and 15.2%, respectively. The lowest population was registered for*Hoplolaimus* spp. with 5.0% (Table 4.8).

Table 4.8 Population Densities of Different Genera of Nematodes in the Soil at the Experimental Site before Application of the Treatments

NematodeSpecies	Number of Nematodes/100ml soil	Percent population density of the nematodes/100ml soil
Hoplolaimus	5.0	0.3
Criconema	10.0	0.6
Tylenchulus	36.0	2.2
Trichodorus	38.0	2.3

Xiphinema	112.0	6.8
Rotylenchus	122.0	7.4
Pratylenchus	251.0	15.2
Helicotylenchus	390.0	23.6
Meloidogyne	686.0	41.6
TOTAL	**1650.0**	**100.0**

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The effect of the organic amendments on chemical properties of the soil at the experimental site in the major and minor seasons is presented in Table 4.9. The application of the organic amendments showed significant increase ($p<0,05$) in organic carbon, organic matter, total nitrogen, potassium and available phosphorus compared to application fertilizer alone, no application and application of carbofuran. However, the application of the organic materials did not show any significant increase ($p>0.05$) between the major and minor seasons in all the parameters (Table 4.9).

Table 4.9 Effect of Organic Amendments on Soil Nutrient Composition after Application of Treatments.2014 Major and Minor Seasons

Treatments	% Organic carbon		% Organic matter		% Total nitrogen		Potassium (mg/kg)		Avail. P (mg/kg)		pH	
	Major season	Minor season	Major season	Minor season	Major season	Minor season	Major season	Minor season	Major season	Minor season	Major season	Minor season
Fresh sweet orange peel (FOP)	1.27d	1.64ab	2.01a	2.10a	0.15bc	0.18a	0.36ab	0.39 a	84.17a	87.27a	6.03c	6.00c
Dry sweet orange peel (DOP)	1.25de	1.38c	1.98a	2.00a	0.14bc	0.17a	0.36ab	0.39 a	77.55ab	83.51a	6.09c	6.00c
Fresh cassava peel (FCP)	1.19de	1.63ab	1.92a	1.98a	0.14bc	0.16ab	0.29cd	0.33bc	57.55cd	60.11cd	6.36bc	6.24bc
Dry cassava peel (DCP)	1.18e	1.61ab	1.86a	1.90a	0.13cd	0.16ab	0.30cd	0.34bc	56.70cd	59.33cd	6.41bc	6.43bc
FCP+FOP	1.22de	1.66ab	1.95a	2.00a	0.14b	0.16ab	0.32bc	0.36ab	73.49ab	79.15ab	6.25bc	6.21bc
FOP +FCP	1.21de	1.68a	1.94a	2.01a	0.14bc	0.16ab	0.33bc	0.37ab	75.96ab	80.32a	6.62ab	6.23bc
DOP+DCP	1.18de	1.60b	1.85a	1.92a	0.14bc	0.16ab	0.30cd	0.37ab	67.61bcd	75.12ab	6.62ab	6.30bc
DCP +DOP	1.19de	1.59b	1.85a	1.91a	0.14bc	0.16ab	0.31cd	0.33b	69.14bc	76.71ab	6.59ab	6.29bc
NPK+ $(NH_4)_2SO_4$ fertilizers	1.02f	0.90f	1.35b	1.33b	0.11de	0.13cd	0.28d	0.32bc	52.94de	55.14cde	6.29bc	6.25bc
Carbofuran	1.01f	0.90f	1.35b	1.33b	0.11de	0.13cd	0.27de	0.32bc	52.66de	55.10cde	6.30bc	6.26bc
No application (control)	1.02f	0.90f	1.35b	1.33b	0.09e	0.08e	0.25de	0.23e	48.61e	46.64e	7.03a	6.99a
LSD	**0.08**		**0.35**		**0.03**		**0.05**		**16.8**		**0.55**	
CV %	**1.1**		**4.8**		**4.6**		**5.1**		**4.7**		**5.6**	

The application of fresh and dry sweet orange peels resulted in the highest increment of all the nutrients in the soils than the cassava peels (Table 4.9). However, on the contrary, no application (control) recorded the highest soil pH in both seasons and the lowest was observed where fresh and dry sweet orange peels were applied (Table 4.9).

4.7.4.2 Growth Parameters of Tomato in the Major and Minor Seasons

The effects of the application of treatments on the fresh shoot weight of tomato in the major and minor seasons are presented in Table 4.10. The combined application of FOP and FCP significantly increased ($P<0.05$) the fresh shoot weight of tomato(374g/plant) in both seasons than the rest of the treatments except the combined application of FCP and FOP. Similarly, all the other treatments were significantly different ($P<0.05$) from the no application in all the seasons. However, there were no significant differences ($P>0.05$) between carbofuran, combined application of NPK and $(NH_4)_2SO_4$, DCP alone, FCP alone and DOP alone in the weight of fresh shoot of tomato (Table 4.10). The fresh shoot weight was found to increase in the application of all the treatments except DCP alone, application of NPK and $(NH_4)_2SO_4$ and no application (Table 4.10).

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Table 4.10 Means of Fresh Shoot Weight, Fresh Root Weight and Stem Girth of Tomato under Different Organic Amendments.2014 Major and Minor Seasons.

Treatments	Fresh shoot wt (g)/plant		Fresh root wt. (g)/plant		Stem girth (cm)	
	Major season	**Minor season**	**Major season**	**Minor season**	**Major season**	**Minor season**
Fresh sweet Orange Peel (FOP)	358.5bc	360.3bc	34.1d	31.2ef	1.0a	1.0a
Dry sweet Orange Peel (DOP)	340.2bcd	365.2bc	31.7ef	29.3fg	1.0a	1.0a
Fresh Cassava Peel (FCP)	337.6cd	355.7bc	34.5de	29.0fg	0.9ab	0.9ab
Dry Cassava Peel (DCP)	319.9d	304.4d	37.6cd	41.4bc	0.9ab	0.9ab
FCP+FOP	358.6bc	372.1ab	30.0f	26.4g	1.0a	1.0a
FOP + FCP	374.0ab	398.5a	28.2fg	25.9g	1.0a	1.0a
DOP +DCP	350.6bc	362.6bc	30.9f	31.2ef	0.9ab	0.9ab
DCP + DOP	347.6bc	357.1bc	30.8f	30.0f	1.0a	0.9ab
NPK+ $(NH_4)_2$ SO_4	313.1d	289.7e	41.3bc	45.7a	0.9ab	0.8bc
Carbofuran	330.5cd	344.9bcd	30.7ef	30.3ef	0.9ab	0.9ab
No application (control)	207.6f	103.2g	42.1ab	43.7ab	0.8bc	0.6c
LSD (p<0.05)	**26.5**		**3.2**		**0.11**	
CV (%)	**5.5**		**3.3**		**4.2**	

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$

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The fresh root weight/plant wassignificantly higher ($P<0.05$) in the no application (control) and application of NPK and $(NH_4)_2SO_4$ fertilizers than the rest of the treatments tested in the major and minor seasons (Table 4.10). However, the fresh root weight in the application of NPK and $(NH_4)_2SO_4$ fertilizers and no application were not significantly different ($P>0.05$)from each other in the major and minor seasons ($P>0.05$).Also, the fresh root weight in the application of FOP alone was not significantly different ($P>0.05$) from FCP alone (Table 4.10) in the major and minor seasons.

The effect of organic amendments on stem girth of tomato in the major and minor seasons is presented in Table 4.10. The highest mean of stem girth was obtained in the application of FOP alone, DOP alone, combined application of FOP and FCP, combined application of FCP and FOP and combined application of DCP and DOP in both seasons. The stem girth of tomato was not significantly different ($P>0.05$) from each other in all the treatments except in the no application in the major and minor seasons (Table 4.10).

4.6.4.3 Yield and Yield Components of Tomato under Different Organic Amendments

The application of FOP and FCPcombination increased the fruit size more than the rest of the treatments in the major and minor seasons (Table 4.11). However, no significant differences (P>0.05) were observed between the fruit size in the combinedapplication of FOP and FCP and all the other treatments except no

application plot in the major and minor seasons. Also, the fruit size observed in all the other treatments were significantly different (P<0.05) from the no application in both seasons (Table 4.11).

Plate 4.2 Fruit Sizeof Tomatoin the Application of Fresh Sweet Orange and Cassava Peels Combination(A) and no Application(B)

The effect of the treatments on the number of fruits/plant of tomato grown in the major and minor seasonsis shown in Table 4.11. The application of NPK and $(NH_4)_2SO_4$

fertilizers and application of dry cassava peel alone significantly increased ($P<0.05$) the number of fruits more than the combined application of FOP and FCP, carbofuran, combined application of FCP and FOP, FOP alone and no application (control) in the major season (Table 4.11). Similarly, the number of fruits/plant was significantly higher than the combined application of FOP and FCP, carbofuran and combined application of FCP and FOPin the minor season. However, the number of fruits/plant in the application of NPK and $(NH_4)_2SO_4$ fertilizers was not significantly different ($P>0.05$) from the application of DCP alone, FCP alone and DOP alone in the minor season. Generally, the number of fruits/plant in all the treatments tested were significantly reduced ($P<0.05$) in the minor season.

The effect of organic amendments on the height of the tomato plants in the major and minor seasons are presented in Table 4.11. The plant height in all the other treatments significantly increased ($P<0.05$) more than no application (control) in the major and minor seasons. However, all the other treatments were not significantly different ($P>0.05$) from each other (Table 4.11) in both seasons.

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Table 4.11 Effect of Treatments on Fruit Diameter, Number of Fruits, Plant Height and Yield of Tomato During 2014 Major and Minor Seasons

Treatments	Fruit diameter (cm)		Number of fruits/plant		Plant height at harvest (cm)		Yield kg/ha	
	Major season	Minor season	Major season	Minor season	Major season	Minor season	Major season	Minor season
FOP	5.8ab	5.5bc	28c	23e	94.4a	94.7a	19212b	15289hij
DOP	5.0d	4.7de	30ab	26cd	93.0a	93.9a	18172bcd	14373j
FCP	4.6de	4.5e	32ab	27cd	90.3a	93.7a	17508cde	14660ij
DCP	4.5e	4.3ef	33a	26cde	91.1a	86.4b	17476def	12394k
FCP +FOP	6.0a	5.8ab	29bc	23e	93.0a	95.0a	20774a	16170fgh
FOP + FCP	6.1a	5.9a	29bc	24e	94.9a	95.5a	20981a	16707efg
DOP +DCP	5.4bcd	5.0de	29bc	23e	93.1a	92.3a	18832bc	14057j
DCP + DOP	5.1d	4.9d	30ab	24e	92.3a	92.6a	18547bcd	14008j
NPK+ ((NH4)2 SO4) fert.	4.0f	4.3ef	33a	28c	93.9a	84.8b	15926ghi	9943l
Carbofuran	5.7ab	5.2cd	28c	24e	91.5a	93.8a	19066b	15155hij
No application (control)	3.2g	2.6h	17d	11f	76.6c	75.9c	6512m	3311n
LSD (p<0.05)	**0.33**		**3.5**		**4.1**		**1282**	
CV (%)	**3.4**		**4.5**		**2.8**		**6.1**	

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$

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The combined application of FOP and FCP significantly increased (P<0.05) the yield of tomato more than the rest of the treatments except the combined application of FCP and FOP in the major and minor seasons (Table 4.11). Also, the yield of tomato in the application of carbofuran were significantly higher (P<0.05) than the application of NPK and $(NH_4)_2SO_4$ fertilizers and no application (control), but no significant difference (P>0.05) occurred between carbofuran and FOP alone in both seasons (Table 4.11).

4.6.4.4 Gall Index of Root-knot Nematodes, Number of Juveniles Extracted from the Roots and Number of Nematodes from the Soil of Tomato Planted in the Major Season

Theeffects of sweet orange and cassava peels on gall index,number of root-knot nematode eggs, number of juveniles extracted from the roots and number of root-knot nematode juveniles from the soil in both seasons are presented in Table4.12. The result showed significant (P<0.05) interaction between the application of sweet orange and cassava peels and seasons.

B

A

Plate 4.3 Severity of Galls on Tomato Root in the Combined Application of Fresh Sweet Orange and Cassava Peels (A) and no Application (B) in the Field

All the treatments that contained sweet orange and cassava peels had significantly lower ($P<0.05$)gall index than NPK and $(NH_4)_2SO_4$ and no application.The gall

indices and number of eggs of root-knot nematodes were significantly lower ($P<0.05$) in the application of carbofuran and combined application of FOP and FCP compared with the other treatments (Plate 4.3) in the major and minor seasons. However, the gall indices and number of root-knot nematode eggs were not significantly different ($P>0.05$) in the application of carbofuran and combined application of FOP and FCP in all the seasons. Similarly, no significant differences ($P>0.05$) were observed between the application of FOP alone and combined application of DOP and

DCP(Table 4.12).

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Table 4.12Effect of Organic Amendment on Gall Index, Number of Eggs and Juveniles of Root Knot Nematodes Extracted from the Root and Soil of Tomato at the Field During 2014Major and Minor season

	[x] Gall index		% reduction of root galling		[z] No. of eggs/10g root		[z] No. of Juveniles/ 10g root		No. nematodes/ 100ml soil	
Treatments	Major Season	Minor season	Major Season	Minor Season	Major Season	Minor Season	Major Season	Minor season	Major season	Minor Season
Fresh Sweet orange Peel (FOP)	3c	2cd	50.0	71.4	1749ef	1580fg	398ef	248hi	758g	542ij
Dry Sweet orange Peel (DOP)	3c	2cd	50.0	71.4	2119de	1903e	436ef	354efgh	858g	675gh
Fresh Cassava Peel (FCP)	3c	3c	50.0	57.1	2206de	2047e	456de	366efgh	880g	742gh
Dry Cassava Peel (DCP)	4bc	5b	33.3	28.6	2678d	3378c	570d	847c	1217f	1633e
FCP +FOP	2cd	1d	66.7	85.7	1667ef	1473fg	269hi	216i	693gh	425j
FOP + FCP	1d	1d	83.3	85.7	1576f	1203fg	254hi	202i	574ij	325j
DOP + DCP	3c	2cd	50.0	71.4	2051e	1610ef	401efg	311g	775g	633hi
DCP + DOP	3c	2cd	50.0	71.4	2072e	1756ef	412efg	343ef	837g	638hi
NPK +$(NH_4)_2$ SO_4 fertilizers	5b	7a	16.7	0	3032c	4049b	877c	1166b	2139d	3025b
Carbofuran	1d	1d	83.3	85.7	1840e	1086g	209i	143i	616hi	327j
No application (control)	6ab	7a	-	-	3225c	4774a	1095b	1344a	2308c	3350a
LSD (p<0.05)	**0.6**		**-**	**-**	**515.7**		**124.5**		**128.5**	
CV (%)	**7.2**		**-**	**-**	**4.3**		**6.3**		**5.1**	

[z]

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Means followed by different letters in the same column and row are significantly different, according to Tukey"s test a $P<0.05$

[z]All data were back-transformed after transforming using $\sqrt{}$; [x]Galling on scale 0-10 where 0 = no galls and 10 = root system completely galled

% percentage values were calculated over the control

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Also, the number of root-knot nematode juveniles in the roots and soil were significantly reduced ($P<0.05$) in the application of carbofuran and fresh sweet orange peel and fresh cassava peel combination treated plots, compared with the rest of the treatments in the major and minor seasons (Table 4.12). However, carbofuran was not significantly different ($P>0.05$) from the combined application of FOP and FCP in terms of reducing the number of juveniles in the root and soil in both seasons (Table 4.12). Similarly, NPK and $(NH_4)_2SO_4$ and no application plots were not significantly different ($P>0.05$) from each other in the two seasons observed. Comparing the performance of the treatments over the seasons, all the treatments were observed to have a declining trend in the parameters observed except NPK and $(NH_4)_2\ SO_4$, DCP and no application plots (Table 4.12).

4.6.4.5 Residual Effects of Organic Amendments on Plant Growth and Root-knot Nematodes

The residual effects of organic amendments on fresh shoot weight, fresh root weight and stem girth of tomato grown in the third season (next major season) are presented in Table 4.13. The residual effect of combined application of FOP and FCP significantly increased ($P<0.05$) the fresh shoot weight of tomato than the rest of the treatments except the combined application of FCP and FOP (Table 4.13). Similarly, FOP alone was significantly different ($P<0.05$) from carbofuran in terms of fresh shoot weight of tomato. No significant differences ($P>0.05$) were observed between carbofuran, FCP alone, DCP alone and DOP alone. All the other test treatments were significantly different ($P<0.05$) from no application (control) (Table 4.13).

The fresh root weight/plant was significantly increased ($P<0.05$) in the combined application of NPK and $((NH_4)_2\ SO_4)$ fertilizers, followed by no application (control) and DCP alone than all the other treatments (Table 4.13). There were significant

differences (P<0.05) between the combined application of FOP and FCP and the rest of the treatments except where FCP and FOP combination was applied Table 4.13). Carbofuran was not significantly different (P>0.05) from FOP alone, FCP alone, DOP alone and DCP alone (Table 4.13).

For the stem girth, the biggest was observed in the combined application of FOP and FCP(1.0cm), while the smallest stem girth was obtained in the no application (0.6cm). Significant differences (P<0.05) occurred between no application and the rest of the treatments. However, no significant difference (P>0.05) was observed between fresh sweet orange and fresh cassava peels (Table 4.13).

The residual effect of organic amendments on fruit diameter of tomato grown in the major season is presented in Table 4.13. The fruit diameter of tomato significantly increased (P<0.05) in the combined application of FOP and FCP more than the rest of the treatments except in the application of FCP and FOP combination (Table 4.13). Significant differences (P<0.05) were observed between the combined application of FOP and FCP and the rest of the treatments, except the application of FOP alone and where FCP and FOP combination was applied (Table 4.13). Also, the combined application of NPK and $(NH_4)_2SO_4$ fertilizerswas significantly different (P<0.05) from the no application (control). There were no significant differences (P>0.05) between DCP alone, FCP alone and DOP alone (Table 4.13).

For the number of fruits/plant, the highest (33 fruits/plant) was observed in theapplication of DCPalone,followed by FCP alone (32 fruits/plant) (Table 4.13). The lowest number of fruits was obtained in the no application (control) (12 fruits/plant) (Table 4.13). The application of DCP alone was significantly different (P<0.05) from

the combined application of FOP and FCP, no application, FOP alone and combined application ofFCP and FOP.

No significant differences ($P>0.05$) were observed between DCP alone, combined application of NPK and ($(NH_4)_2 SO_4$) fertilizers, FCP alone, DOP alone and carbofuran (Table 4.13).

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Table 4.13 Residual Effect of Organic Amendments on Plant Growth and Yield of Tomato in the Field. 2015 Major Season.

Treatments	Fresh shoot weight (g)/plant	Fresh root weight (g)/plant	Stem Girth (cm)	Fruit diameter (cm)	Number of fruits/plant	Plant height at harvest (cm)	Yield kg/ha
Fresh sweet Orange Peel (FOP)	333.4ab	32.1de	0.9b	4.8b	29.0c	91.3a	16582.6 b
Dry sweet Orange Peel (DOP)	298.6cd	34.2cd	0.9b	4.3bc	31.0abc	89.7ab	16074.5 b
Fresh Cassava Peel (FCP)	273.5cd	34.8c	0.9b	4.0c	32.0ab	89.2ab	15500.3 d
Dry Cassava Peel (DCP)	257.7d	42.3b	0.8c	3.3d	33.0a	85.5bc	13001.0 e
FCP+FOP	321.1ab	30.4ef	0.9b	5.1a	28.0c	92.1a	17088.2 a
FOP+ FCP	358.2a	29.5f	1.0a	5.3a	27.0c	94.0a	17387.3 a
DOP +DCP	326.4ab	33.2cd	0.9b	4.7b	29.0c	91.1a	16386.0 b
DCP+ DOP	310.8bc	33.3cd	0.9b	4.5b	30.0bc	90.4a	16269.1 bc
NPK+ $(NH_4)_2$ SO_4 fertilizer	211.3e	46.1a	0.8c	3.0d	33.0a	84.0c	11958.2 e
Carbofuran	272.6cd	33.7cd	0.9b	4.2bc	31.0ab	89.3ab	15713.0 cd
No application (control)	98.2f	44.2ab	0.6d	2.1d	12.0d	72.2d	2983.5 f
LSD (p<0.05)	**38.9**	**3.3**	**0.1**	**0.7**	**2.9**	**4.8**	**509.1**
CV (%)	**10.5**	**4.9**	**10.7**	**7.8**	**8.6**	**4.0**	**2.8**

Means followed by different letters in the same column are significantly different, according to Tukey‟s test at *P*<0.05

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The highest plant height (94.0cm) at harvest was recorded in the combined application of FOP and FCP, whilst the lowest plant height was observed in the no application (72.2cm). The height of plants recorded under the organic amendments and carbofuran were significantly different ($P<0.05$) from no application. No significant differences ($P>0.05$) were observed between the organic amendments and carbofuran in terms of plant height at harvest (Table 4.13).

The residual effect of thecombined application of FOP and FCPsignificantly increased ($P<0.05$) yield of tomato compared with the other treatments (Table 4.13). Significant differences ($P<0.05$) were observed between the combined application of FOP and FCP and the rest of the treatments except where FCP and FOPcombination was applied (Table 4.13). No significant differences ($P>0.05$) occurred between carbofuran, FOP alone and DOP alone. Similarly, no significant difference ($P>0.05$) was observed between FOP alone and DOP(Table 4.13).

4.6.4.6 Relationship Between Yield and Yield components of Tomato

Table 4.14 shows the correlation analysis of fruit diameter, number of fruits and yield of tomato. Significant positive ($p<0.05$) correlation was observed between the fruit diameter and fresh fruit yield of tomato. However, there was no significant relationship ($p>0.05$) between the number of fruits and yield of tomato. Similarly, a very weak relationship was observed between fruit diameter and size of tomato (Table 4.14).

Table 4.14 Relationship Between Fruit Diameter, Number of Fruits and Yield of Tomato

	Fruit diameter	No .of fruits
Fruit diameter	-	-
No. of fruits 0.2344 - **Yield/ha** 0.8118 0.3737		

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4.6.4.7Residual Effects of Organic Amendments on Gall Index, Number of Eggs and Number of Nematodes Extracted from the Roots and Soil of Tomato in the Major Season

The gall index scored on roots of tomato after harvest varied from 3 to 8.5 (Table 4.15). The highest gall index (8.5) was recorded in the no application (control), followed by the combined application of NPK and ($(NH_4)_2$ SO_4) fertilizers (8.3).

Galling was significantly reduced ($P<0.05$) by 64.7% in the combined application of FOP and FCP and the application of FCP and FOPcombinationcompared to the rest of the treatments (Table 4.15). There were significant differences ($P<0.05$) between the combined application of FOP and FCP and the rest of the treatments except where FCP and FOP combination was applied. Similarly, FOP alone was significantly different ($p<0.05$) from FCP alone, DOP alone and DCP alone. No significant difference ($P>0.05$) occurred between no application (control) and combined application of NPK and ($(NH_4)_2SO_4$) fertilizers (Table 4.15).

The residual effect of the treatments on the number of root-knot nematode eggs/10g root of tomato in the major season ranged from 1180 to 2571 eggs/10g root. The combined application of FOP and FCP significantly reducedthe number of root-knot nematode eggs more than the other treatments tested. The highest number of root knot nematode eggs was recorded in the no application (Table 4.15).

Table 4.15Residual Effect ofthe Treatments on Gall index, Number of eggs andNumber of nematodes from the Root and Soil of Tomato in 2015 Major season

Treatments	[x] Gall index	% reduction of root galling	[z] Number of eggs/10g root	[z] Number of juveniles/10g soil	[z] Number of nematodes /100ml root
Fresh Orange Peel (FOP)	3.5e	58.8	1294.0f	214.0bcd	429.0e
Dry Orange Peel (DOP)	4.8f	43.5	1809.0d	231.0bc	493.0d
Fresh Cassava Peel (FCP)	6.8d	20.0	2059.0c	260.0b	528.0c
Dry Cassava Peel (DCP)	7.5c	11.8	2182.0b	273.0ab	663.0 b
FCP + FOP	3.0h	64.7	1211.0fg	169.0d	385.0f
FOP + FCP	3.0h	64.7	1180.0g	161.0d	340.0f
DOP +DCP	3.8g	55.3	1678.0 e	191.0cd	463.0de
DCP + DOP	4.3f	49.4	1711.0de	202.0cd	474.0de
NPK+ $(NH_4)_2$ SO_4fertilizers	8.3b	2.4	2557.0a	308.0ab	1231.0 a
Carbofuran	5.5e	35.3	1875.0d	257.0b	451.0de
No application (control)	8.5a	-	2571.0a	314.0a	1263.0a
LSD (p<0.05)	**0.1**	**-**	**48.6**	**41.7**	**44.9**
CV (%)	**9.1**	**-**	**3.4**	**9.7**	**6.4**

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$ [z] All data were back-transformed after transformation using $\sqrt{}$

[X]Galling on scale 0-10 where 0 = no galls and 10 = root system completely galled

The number of juveniles extracted from the roots of tomato in the major season ranged from 161 to 314 juveniles/10g root (Table 4.15). The number of juveniles extracted from the roots was significantly reduced (P<0.05) in the combined application of FOP and FCP more than the rest of the treatments. The highest number of root-knot nematode juveniles was extracted in the no application (Table 4.15).

Significant differences ($P<0.05$) occurred between the application of FOP and FCP combination and the rest of the treatments except the combined application of FCP and FOP. Carbofuran was not significantly different ($P>0.05$) from the combined application of NPK and$(NH_4)_2SO_4$ fertilizers. Similarly, fresh sweet orange peel alone was not significantly different ($P>0.05$) fromDOP (Table 4.15).

The combined application of FOP and FCPsignificantly reduced the population of root-knot nematodes in the soil more than all the other treatments (Table 4.15). The highest root-knot nematode population extracted from the soil was observed in the no application. Significant differences ($P<0.05$) were observed between the combined application of FOP and FCP and the rest of the treatments (Table 4.15). However, carbofuran was not significantly different ($P>0.05$) from FOP alone and DOP alone. Also, no significant difference ($P>0.05$) was oberved between no application and combined application of NPK and$(NH_4)_2SO_4$ fertilizers (Table 4.15).

4.6.4.8Effect of the Treatments on Fungi and Free-Living Nematodes in the Soil before and after Application of Treatments

The initial population of fungi, bacterivorous and fungivorous nematodes are presented in Table 4.16. *Colletotrichumgloeosporioides* and *Aspergillusniger*were found to be the most prevalent fungi, while*Heterocephalobellus* sp.and *Ditylenchus*sp. were the most abundant bacterivorousand fungivorous nematodes, respectively, before the application of the treatments (Table 4.16). The initial population of *T.viride* was found to be low, compared with other fungi, before application of amendments
(Table 4.16).

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Table 4.16Fungi and Free-Living Nematodes Isolated from the Experiment before Treatment Application

No. of fungal colonies/1g soil				No. of bacterivorousnematodes/100ml soil		No. of fungivorous/100ml nematodes soil
T. viride	*C.gloeosporioides*	*P. chrysogenum*	*A. niger*	*Heterocephalobellus* sp.	*Eucephalobus*sp.	*Ditylenchus*sp.
2	4	3	4	36	32	44

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4.6.4.9Number of Fungi and Free-Living Nematodes in the SoilAfter Addition ofSweet Orange and Cassava Peels

The number of colonies of biocontrol fungi, number of bacterivorous and fungivorous nematodes extracted from the soil after the application of the different treatments are presented in Table 4.17. The types of microorganisms predominantly found in the soil were *T. viride*, a nematode biocontrol fungus and other fungi (*C. gloeosporioides,P. chrysogenum* and *A. niger*), *Hetetocephalobellus* sp.and *Eucephalobus* sp. as bacterivorous nematodes and *Dictylenchus* sp. as fungivorous nematode were also found after application of the treatments (Table 4.17). The combined application of FOP and FCP significantly increased ($P<0.05$) the number of colonies of *T. viride*, followed by the application of fresh sweet orange peel alone (Table 4.17). The smallest number of colonies was observed in the carbofuran treated plots(Table 4.17). In the case of bacterivorous and fungivorous nematodes, the highest populations were observed where fresh and dry cassava peels were applied, compared with fresh and dry sweet orange peels and their combinations with cassava peels (Table 4.17). The bacterivorous and fungivorous nematodes extracted from the soil after application of the treatments were found to be significantly fewer in plots treated with carbofuran, fresh and dry sweet orange peels. Also, the combination of sweet orange and cassava peels had fewer

populations of bacterivorous and fungivorous nematodes, compared with FCP alone or DCP alone (Table 4.17).

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Table 4.17 Fungi and free-living nematodes isolated from the soil after treatment application

Treatment	Number of fungal colonies/1g soil				Number of bacterivorous nematodes/100ml soil		Number of fungivorous nematodes/ 100ml soil
	T. viride	*C. gloeosporioides*	*P. chrysogenum*	*A. niger*	*cephalobellus* sp.	*Eucephalobus* sp.	*Ditylenchus* sp.
Fresh Sweet orange Peel (FOP)	8 a	2 c	2 bc	2 b	4 c	4 b	8 b
Dry Sweet orange Peel (DOP)	6 a	1 c	2 bc	2 b	0 c	4 b	4 b
Fresh Cassava Peel (FCP)	2 b	4 ab	3 ab	5 a	96 a	72 a	92 a
Dry Cassava Peel (DCP)	1 b	5 a	3 ab	6 a	68 a	64 a	100 a
FCP +FOP	8 a	2 d	1 c	3 b	32 b	28 b	20 b
FOP + FCP	9 a	3 bc	2 bc	2 b	8 c	8 b	12 b
DOP + DCP	7 a	1 c	2 bc	3 b	4 c	4 b	8 b
DCP + DOP	6 a	2 c	3ab	3 b	20 bc	16 b	24 b
NPK+ $(NH_4)_2$ SO_4fertilizers	2 b	4 ab	4 a	5 a	16 bc	12 b	8 b
Carbofuran	1 b	3 bc	2 bc	2 b	0 c	4 b	0 b
No application (control)	1 b	6 a	2 bc	3 b	12 c	16 b	12 b
LSD (p<0.05)	**2.3**	**2.1**	**1.2**	**1.3**	**26.4**	**28.2**	**24.8**
CV (%)	**6.2**	**8.3**	**3.3**	**3.8**	**7.9**	**10.2**	**9.8**

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*Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$

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EXPERIMENT FOUR

4.7Experiment 4: Potential of *T. viride* and Organic Amendments in Root-knot Nematode Management *in vitro*

4.7.1 Methodology

The experiment was conducted at the plant house,Faculty of Agriculture, KNUST, from 22nd September, to 22nd November, 2015. This experiment was done to validate the results of the field trial on the potential of *T. viride* and its combined application with organic amendments in the managementof root-knot nematodes in tomato. Also, the effect of sweet orange and cassava peels on the sporulation of the fungus was evaluated to confirm the field result.

4.7.2 Experimental Design

The experiment was laid in a Completely Randomized Design with five treatments replicated five times. The following treatments were tested:

1. Fresh orange peels + *T. viride*
2. Fresh cassava peels + *T. viride*
3. *T. viride* alone
4. Carbofuran+ *T. viride*
5. No application (Control)

4.7.3 Experimental Condition

The condition of the plant house and soil were as described at sections 4.5.1.1 and 4.3.7 respectively.

4.7.4 Materials

4.7.4.1 Fresh Sweet Orange and Cassava Peels

The fresh sweet orange and cassava peels were collected from the same source as described in experiment one. The fresh sweet orange and cassava peels were applied

separately at four weeks before transplanting. The application of the peels and carbofuran were done as indicated at sections 4.5.1.6 and 4.5.1.7 respectively.

4.7.4.2 Soil Preparation

A weight of 1.8kg of steam-sterilized soil was placed in 2 L size pots. The soil was prepared as described at section 4.3.7.

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4.7.4.3 Isolation of *T. viride*

The soil used was collected from the research field of the Faculty of Agriculture. *T. viride* was isolated from the soil using Wantanabe (2000) method. The fungus was grown on Potato Dextrose Agar (PDA) using serial dilution at a concentration of 10^{-6}. Asoil suspension of 50µl was evenly spread on the medium (PDA) in each 9cm diameter Petri dish. The Petri dishes were placed on the laboratory bench at about 26 to 28°C for one week for the fungus to grow.

4.7.4.4 Inoculation of the Soilwith the Spores of *T. viride*

The spores were counted, using a haemocytometer.A concentration of 50 x 10^3 spores of *T. viride* (Kerry, 2001) was applied in the soil, using a syringe at four weeks before transplanting. The spores were diluted in 10ml of distilled water in 20ml size beaker and the suspension was stirred for 20 seconds. An aliquot (5µl) of the suspension was placed on a haemocytometer under the compound microscope. All the spores were counted in each of the boxes on the haemocytometer, using a counter.

4.7.4.5 Inoculation of the Soil with Root-knot Nematode Eggs

Eggs were extracted (Hussey and Barker, 1973) from the roots of infected tomato plants grown in pots in the plant house. A total of 2000 eggs of root-knot nematodes was

placed in each pot, using a syringe. The eggs were also inoculated in the steamsterilized soil at four weeks before transplanting.

4.7.4.6 Nursery Preparation and Transplanting of Seedlings

Tomato seeds were sown in steam-sterilized soil in rows at a depth of 3cm and 10cm between rows. At two weeks after emergence, the seedlings were hardened by placing them under the sun for one week before transplanting. The seedlings were transplanted at four weeks after the application of the treatments. One seedling was transplanted in each pot. One hundred millilitres (100ml) of tap water was applied to each plant every two days till harvest.

4.7.5 Data Collected

The following data were collected:

1. **Fresh shoot weight (g):** The shootpart of the plant was cut from the base of the stem and weighed immediately after harvest. Both the stem and leaves were weighed together to represent the fresh shoot weight.
2. **Plant height (cm):** The shoot length was measured from the base of the plant to the tip of the tallest branch. This variable was measured at harvest.
3. **Fresh root weight (g):** The fresh root weight was measured when roots were fresh. The process was done as described at section 4.5.1.16.
4. **Number of juveniles/5g root:** The number of juveniles extracted from roots was counted after harvest. The counting of juveniles was repeated three times and the mean was calculated for each sample.
5. **Number of nematodes/100ml soil:** The number of root-knot nematodes extracted from the soil was counted afterharvest (eight weeks after transplanting).

6. **Number of eggs/5g root:** This variable was determined as described at section 4.5.1.16.
7. **Gall index:**The gall index was determined as described at section 4.51.16.
8. **Reproduction factor:** The reproduction factor was calculated by dividing the final population (nematodes and eggs) by the initial population.
9. **Number of colonies of *T.viride*:** One gramme of soil was weighedand serially diluted (Watanabe, 2000). About 50µl of the suspension was spread on the prepared Potato Dextrose Agar (PDA). The colonies of the fungi grown on PDA were counted one week after inoculation.

4.7.6 Data Analysis`

All the data were analyzed by using ANOVA and means were separated, using Tukey‟s test at $p<0.05$. The variables on nematode count (number of juveniles/5g root, number of nematodes/100ml soil and number of eggs/5g root) were square root transformed $(\sqrt{(x+1)})$, where x is the mean counted before statistical analysis and then back-transformed.

4.8Results

4.8.1 Effect of *T. viride* and Organic Amendments on the Growth Factors of Potted Tomato in the Plant House

The effects of *T. viride* and combined application of *T. viride* and organic amendments on fresh shoot weight, shoot length and fresh root weight are shown in Table 4.18. The fresh shoot weight/plant significantly increased (P<0.05) in the combined application of fresh sweet orange peel and *T. viride* than the rest of the treatments. Significant differences (P<0.05) were observed between the combined application of fresh sweet orange peel and *T. viride* and the rest of the treatments. Similarly, the combined application of fresh cassava peel and *T. viride* was found to be significantly different (p<0.05) from the application of *T. viride* alone and carbofuran. The fresh shoot weight in all the other treatments were found to be significantly different (P<0.05) from the no application treatment (Table 4.18).

Table 4.18Effects of the Treatments on Tomato Growth Factors at Eight Weeks after Transplanting in the Plant House

Treatments	Fresh shoot weight (g)/plant	Shoot length (cm)	Fresh root weight (g)/plant
Fresh sweet orange peel +*T.viride*	24.1a	63.3a	3.1d
Fresh cassava peel+*T. viride*	22.2a	61.5a	3.6c
T. viride alone	17.5b	54.8c	4.0b
Carbofuran+ *T. viride*	18.8b	58.8b	3.2d
No application (control)	11.7c	40.6c	4.9a
LSD (p0<0.05)	**1.9**	**1.9**	**0.79**
CV (%)	**4.1**	**2.4**	**5.3**

Means followed by different letter in the same column are significantly different, according to Tukey"s test at *P*<0.05

The combined application of fresh sweet orange peel and *T. viride* had significantly increased (P<0.05) the fresh shoot weight and length/plant of tomato than the rest of the treatments, followed by the combined application of fresh cassava peel and *T. viride*

(Table 4.18). There were significant differences (P<0.05) between the combined application of fresh sweet orange peel and *T. viride* and the rest of the test treatments except combined application of fresh cassava peel and *T. viride*. Also, the fresh shoot weight and length/plant in the combined application of fresh cassava peel and *T. viride*were significantly different (P<0.05) from *T. viride* alone, combined application of carbofuran and *T. viride* and no application. No significant difference (P>0.05) was observed between *T. viride* alone and combined application of carbofuran and *T. viride* (Table 4.18).

The fresh root weight/plant of tomato significantly increased (P<0.05) in the no applicationthan the rest of the treatments (Table 4.18). Similarly, the fresh root weight in the combined application of fresh cassava peel and *T. viride,* fresh sweet orange peel and *T. viride*combination and *T. viride* alone were significantly different (P<0.05) from each other. However, the fresh root weight in the combined application of fresh sweet orange peel and *T. viride* was not significantly different (P<0.05) from the combined application of carbofuran and *T. viride* (Table 4.18).

4.8.2 Effect of *T. viride* and Organic Amendments on Gall Index and Number of Root-knot Nematodes in the TomatoRootsand Soil

The gall index of root-knot nematodes was significantly greater (P<0.05) in the no application than all the other treatments (Table 4.19). Galling was significantly reduced (P<0.05) in the combined application of fresh sweet orange peel and *T. viride* (83.8%) compared to the no application. Also, gall index under the combined application of fresh cassava peel and *T. viride*(70.3 %) and the application of *T. viride* alone (40.5 %) were significantly smaller (P<0.05) than no application (Table 4.19).

The number of eggs extracted from the roots of tomato was significantly lower (P<0.05) in the combined application of fresh sweet orange peel and *T. viride* than the rest of the

treatments (Table 4.19). The combined application of fresh sweet orange peel and *T. viride*, combined application of fresh cassava peel and *T. viride* and combined application of carbofuran and *T. viride* were found to be significantly different (P<0.05) from the application of *T. viride* alone and no application. However, the number of eggs in the combined application of fresh sweet orange peel and *T. viride*, combined application of fresh cassava peel and *T. viride* and combined application of carbofuran and *T. viride* were not significantly different (P>0.05) from each other. Similarly, *T. viride* alone was found to be significantly different (P<0.05) from the no application for the number of eggs (Table 4.19).

Table 4.19 reveals the effect of *T. viride* and organic amendments combined on the number of juveniles in the root and soil at eight weeks after transplanting. The combined application of fresh sweet orange peel and *T. viride* significantly reduced (P<0.05) the number of juveniles of root-knot nematodes in the roots of tomato than the rest of the treatments, followed by combined application of carbofuran and *T. viride*. The number of juveniles of root-knot nematodes extracted from the root in all the other treatments applied were found to be significantly different (P<0.05) from the no application. No significant difference (P>0.05) was observed between the combined application of fresh cassava peel and combined application of carbofuran and *T. viride* in terms of reducing the number of root-knot nematodes in the roots (Table 4.19).

Table 4.19 Effect of T. viride and Organic Amendments Combination on the GallIndex, Number of Eggs, Number of Juveniles Extracted from the Rootsand Soilof Tomato in the Plant House

Treatments	[x]Gall index	% galling reduction	[z]No. of eggs/5g root	[z]No. of Juveniles/ 5g root	[z]No. of Nematodes/ 100ml soil	[y] Reproduction factor
FOP +*T. viride*	1.2d	83.8	668.0c	153.0d	340.0e	0.3

FCP+*T. viride*	2.2c	70.3	784.0c	488.0c	580.0c	0.5
T. viride alone	4.4b	40.5	1855.0b	858.0b	1060.0b	1.0
Carbofuran + *T. viride*	1.6cd	78.3	777.0c	365.0c	430.0d	0.4
No application (control)	7.4a	-	3303.0a	1272.0a	2430.0a	1.9
LSD (p<0.05)	**0.8**	**-**	**133.4**	**129.5**	**131.3**	
CV (%)	**7.3**	**-**	**4.2**	**10.0**	**6.8**	

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$ [z] All data were back-transformed after transformation using $\sqrt{\quad}$

[x]Galling on scale 0-10 where 0 = no galls and 10 = root system completely galled (Bridge and Page, 1980) [y] Reproduction factor = Final population (Pf)/Initial population (Pi), where Pi = 2000

The combined application of fresh sweet orange peel and *T. viride* significantly lowered (P<0.05) the number of root-knot nematodes in the soil than all the other treatments tested (Table 4.19). Significant differences (P<0.05) occurred between the combined application of fresh sweet orange peel and *T. viride* and the rest of the test treatments except in the combined application of carbofuran and *T.viride.* Also, the combined application of fresh cassava peel and *T. viride* and *T. viride* alone were found to be significantly different (P<0.05) from the no application for the number of root-knot nematodes from the soil (Table 4.19).

The reproduction factor of root-knot nematodes ranged from 0.3 to 1.9. The reproduction factor was observed to have highly reduced in the combined application of fresh sweet orange peel and *T. viride* than the rest of the treatments, followed by the combined application of carbofuran and *T. viride*. The reproduction factors in the application of *T. viride* alone and no application were 1 and 1.9, respectively. Thus *T.viride* alone is not that effective for root-knot nematode management (Table 4.19).

4.8.3 Effect of theTreatments on the Reproduction of *T. viride* at Eight Weeks after Transplanting of Tomato

Table 4.20 shows the number of colonies of *T. viride* under different treatments tested in the plant house. The highest number of colonies was counted in the combined application of fresh sweet orange peel and *T. viride*, followed by the combined application of fresh cassava peel and *T. viride*. The lowest of number of colonies of *T. viride* was found in the application of carbofuran and *T. viride* combination (Table 4.20).

Table 4.20Effect of the Treatments on the Number of Colonies of T. viride in thePlant House

Treatments	**No. of colony**
Fresh sweet orange peel +*T. viride*	11.0a
Fresh cassava peel+*T. viride*	7.0b
T. viride alone	6.0b
Carbofuran + *T. viride*	4.0b
No application (control)	0.0c
LSD (p<0.05)	**3.5**
CV (%)	**17.1**

Means followed by different letters in the same column are significantly different according to Tukey"s test at *P*<0.05

There were significant differences (P<0.05) among the test treatments (Table 4.20). Significant differences (P<0.05) were observed between the combined application of fresh sweet orange peel and *T. viride* and the rest of the treatments. However, the combined application of fresh cassava peel and carbofuran and *T. viride*combination and *T. viride* alone were not significantly different (P>0.05) from each other (Table 4.20).

4.9Discussion

The evaluation of the efficacy of organic soil amendment in the management of rootknot nematodes involved different experiments.The results of the different parameters collected at the laboratory, plant house and the field showed highly significant differences. At the laboratory, aqueous extracts of fresh orange peel significantlyinhibited egg hatching inhibition and increased mortality of root-knot nematodes than the rest of the test treatments. This may be attributed to the limonene in the fresh orange peels. According to Isman *etal*. (2008),lemonene in sweet orange peels has shown biological activity against a wide range of plant pests with effects on the growth rate and reproduction of plant-parasitic nematodes. Similar experiment carried out in the laboratory by Tsai (2008) showed that aqueous extract of orange peels inhibited egg hatching of root-knot nematodes by 91% and caused mortality of juveniles by 93.5% after 48 and 72h of exposure, respectively.

In addition, the higher efficacy of sweet orange peels over the cassava peels in the inhibition of egg hatching and juvenile mortality may be attributed to higher stability of lemonene compared to hydrogen cyanide. Li and Chang (2011) stated that lemonene moderately dissolved in water and remained stable for eight weeks after storage, while hydrogen cyanide had low persistence in water. John *et al.* (2009) reported that the toxicity of phytochemicals was related to the concentration of active compounds and the time of exposure of the nematodes to the products.

Similarly, aqueous extracts of fresh and dry cassava peels and dry orange were found to inhibit egg hatching and kill root-knot nematode juveniles. The effect of the organic amendments on egg hatching can be attributed to the permeability of the eggs as reported by Perry *et al.* (2009). When eggshells are permeable, the unhatched second stage juveniles are susceptible to toxic compounds, including plant extracts that may have the potential as control agents (Perry *et al.,* 2009).

In comparing the fresh peels and dry peels, it was observed that fresh peels of orange and cassava had higher egg hatching inhibition and mortality compared with the dry peels. The higher inhibition effect on egg hatching and mortality of juveniles by the fresh peels may be due to higher concentration of the phytochemicals (lemonene and hydrogen cyanide) in the peels. Studies have shown that concentration of hydrogen cyanide is higher in the fresh peels, compared to the dry peels of cassava. Dhas *et al.* (2011) indicated that concentration of hydrogen cyanide is higher in the fresh peel of cassava (364.2 – 814.7ppm) than dry peel (264.3 – 321.5ppm). Similarly, Kamal *et al.* (2011) reported that concentration of lemonene was found to be significantly higher in the fresh peel of sweet orange (80.9%), compared to dry peels (66.8%).

In general, the peels of orange and cassava caused highest mortality of root-knot nematodes juveniles at 48h of exposure. This result is in agreement with Tsai (2008) who reported that aqueous extract of fresh orange peels showed significant mortality of juveniles of *M. incognita* at 48h of exposure in the laboratory. *In vitro* studies showed significant mortality of *M.incognita* second stage juveniles due to toxic effect of hydrogen cyanide from dry cassava peels (John *etal.*, 2009).

In the case of the experiment carried out in the plant house, poultry manure was observed to have significantly increased shoot length, root length and fresh shoot weight of tomato more than the other treatments tested. This could be due to the higher concentration of nutrients such as nitrogen in poultry manure that induced growth and development of the plant. Laboratory analysis of nutrients in poultry manure showed higher amount of total nitrogen compared to the other organic amendments tested. This result is in agreement with that of Meyer *et al*. (2011) who indicated significant increase in plant height, leaf area, shoot dry weight, stem girth and root dry weight of cacao plants with application of poultry manure.

However, poultry manure did not have significant effect in reducing the number of juveniles in the root and soil and number of eggs of root-knot nematodes compared with fresh orange and cassava peels. The fresh orange peel, although significantly different from carbofuran had lower number of juveniles extracted from the root and soil, number of eggs, gall index and reproduction factor compared with fresh cassava, dry cassava, dry sweet orange peels and poultry manure at the plant house. The significant reduction of juveniles and eggs of root-knot nematodes in the application of fresh orange peel might be attributed to higher presence of lemonene. According to Li *et al*. (2013), essential oil of sweet orange peels in which lemonene is one of the major constituents exhibited strong nematicidal activity against *M. incognita*, with a LC_{50} value of 47.3µg/ml.

Generally, fresh sweet orange and cassava peels performed best in reducing gall index, number of eggs and population of root-knot nematodes, compared with the dry peels. This can be due to the higher concentration of the phytochemical responsible for nematode suppression in the fresh peels. The result of the laboratory analysis of the cyanide content in fresh and dry peels of cassava showed higher concentration of cyanide in the fresh cassava peels, compared to dry peels. Also, the dry sweet orange and cassava peels were found to be better than poultry manure and rice husk in reducing the number of juveniles in the root, number of nematodes in the soil, number of eggs and gall index. This significant reduction of root-knot nematodes population in the roots and soil and gall index by dry sweet orange and cassava peels can be related to the effectiveness of phytochemicals in peels, compared to rice husk and poultry manure. The plant house experiment carried out by Loumédjinon *et al* (2007) indicated that dry sweet orange and cassava peels significantly reduced the number of root-knot nematodes in soil and roots and percentage roots galled and increased number of roots

and biomass of carrot, compared to control. Also, John *et al.,* (2009) reported significant reduction of the second stage juveniles of *M. incognita* in the application of dry cassava peels more than poultry manure.

The rice husk was not significantly different from control (no application) in terms of reducing the number of juveniles in the roots and soil, number of eggs, gall index and reproduction factor. However, the results obtained from this experiment is contrary to that of Chakrabarti *et al*. (2015) who stated that rice husk in the form of biochar releases certain organic compound such as phenols and furfurals, which have potential toxic effect on nematodes. The differences in preparation of organic amendments (fresh or dry; composted or not) and application methods can influence the effectiveness of the material used (Oka, 2010). Therefore, the ineffectiveness of rice husk could be due to the method of preparation or form used, which might have caused poor decomposition for eventual nematode suppression or mortality.

For the field experiment, significant increase of soil nutrient content was recorded after application of organic amendments in the major and minor seasons. The fresh and dry sweet orange peels were observed to increase the soil organic carbon, organic matter, total nitrogen, potassium and phosphorus contents more than the rest of the organic amendments. This might be due to the high contents of these elements in fresh and dry sweet orange peels, compared with the other amendments. Also, the nutrient levels observed in the application of fresh and dry cassava peels were found to be higher than the control, inorganic fertilizer (NPK and $(NH_4)_2SO_4$ combined) and application of carbofuran. The higher content of the nutrients in the organic amended plots might be related to the high amount of organic matter content which had helped to retain the nutrients by preventing them from leaching. FAO (2005) reported that organic matter increase cation exchange capacity and nutrient retention in the soil.The incorporation

of organic amendments in the soil increasesabsorption of nutrient elements and decrease nutrient loss in the soil through leaching(Brown *et al*., 2005).

On the plant growth parameters, the combined application of fresh sweet orange and cassava peels significantly increased fresh shoot weight, fruit diameter and plant height of tomato more than the rest of the test treatments in all the three experiments. This can be explained by the improved soil condition in terms of organic matter content, nutrient supply, low root-knot nematode population and higher number of colonies of *T. viride* observed under the treatment. According to Karmani *et al*. (2011), application of neem seed cake and animal waste significantly reduced the population and reproduction of root-knot nematodes and concomitantly increased growth and development of eggplant at the field. Also, Zasada *et al*. (2003) reported that composted municipal wastes and sludges have been used to amend soil to improve soil fertility, organic matter content, water holding capacity, nutrient retention, and cation exchange capacity of the soil. Similarly, Overstreet and DeJong-Huges (2006) stated that organic matter retains plant nutrients and prevents them leaching to deeper soil layers. According to Okonkwo*etal.* (2011) application of cassava peels increased yield of maize.

The fresh root weight observed under the various treatments was significantly higher in the control (no application) compared with the rest of the treatments. The higher fresh root weights in the control could be attributed to the effect of the size and number of galls on the biomass of the roots as a result of increased root-knot nematode population. Hussain *et al.* (2011) reported significant increase in the number of galls in plots with higher number of egg masses and nematode population. Also, Corbett *et al*. (2011) observed a positive relationship between root biomass of okra and number of root-knot nematodes in the root and soil. There was an increase in root biomass of okra as the population of root-knot nematode increased (Corbett *et al*., 2011).

The application of the various treatments had significant effect on the fruit size and yield of tomato. The largest fruit yield was observed in the combined application of fresh sweet orange and cassava peels in all the seasons observed. The largest fruit yield in the combined application of fresh orange and cassava peels might be attributed to the low root-knot nematode population. Corbett *et al*. (2011) indicated that nematode infection at higher inoculum dramatically reduced the number, size and total weight of mature fruits produced by susceptible tomato plants. Similarly, Bawa *et al.* (2014) reported that at higher number of root-knot nematodes, the yield of tomato was significantly reduced. Perry *etal*. (2009) reported that the level of reduction in quantity and/or yield of crops due to root-knot nematode damage is positively correlated to their population. Also, the sweet orange and cassava peels contained appreciable amount of nutrients (organic carbon, total nitrogen, potassium and phosphorus), which could have contributed to the increase in yield of tomato in the combined application of fresh sweet orange peel and fresh cassava peel compared to carbofuran.

With regard to the effect of organic amendments on the population of root-knot nematodes, the combined application of fresh sweet orange and cassava peels significantly reduced the number of juveniles in the root and soil, gall index and number of eggs as good as carbofuran in the first major and minor seasons. The low root-knot nematode population and damage under this treatment could be due to the combined effect of the nematicidal properties in the two peels. The application of fresh sweet orange peels first might have significantly reduced the initial population of root-knot nematodes and by the time the population started to increase again, the additional application of fresh cassava peels at three weeks after transplanting could have reduced their number significantly below that of the other treatments. This result agrees with that of Sundararaju and Kumar (2012) who reported that the integration of organic,

inorganic and green manure was more effective in reducing the number of nematodes and subsequently increased plant growth and yield than the organic treatment alone. In a similar research, Ram *et al*. (2009) reported that combination of neem, mustard and castor cakes as soil amendments significantly reduced population of root-knot nematode (*M. incognita*) and improved plant growth parameters of okra. Hussain *etal*. (2011) found the population density of root-knot nematodes and galling to be significantly lowered in the combined application of neem leaves and marigold, compared with sole treated amended plots on tomato.

In the third experiment (major season), where no application of treatments was made, some increase in the number of root-knot nematodes in the root and soil was observed. However, the plots treated with a combination of fresh sweet orange and cassava peels recorded significantly smaller numbers of root-knot nematodes in the root and soil, gall index and number of eggs than the rest of the treatments. This might be attributed to the smaller population density of root-knot nematodes at the time of transplanting (initial population) and already well-established amount of *T. viride* in those plots. The colony count of *T. viride* observed in the plots treated with both fresh sweet orange and cassava peels was higher than in all the other treatments. This might be due to the presence of sweet orange peels, which contain certain chemical compounds that favour growth and multiplication of *T. viride*, compared to the other treatments (cassava peels, NPK and $(NH_4)_2SO_4$ combined and carbofuran). According to Duli, *et al.* (2013),*T. harzianum* showed highest growth rate and sporulation on sweet orange peel substrate compared with other substrates tested. Previous research showed that *Trichoderma* spp. have good potentials for plant parasitic nematode control. Khan *et al*. (2010) reported that *T. harzianum* decreased the negative effects of nematodes, leading to a decrease in galling and an enhancement in the growth and yield of eggplant. The soil application

of *T. harzianum* significantly improved the dry shoot weight 6-7% over the control (Khan *et al*., 2010). Also, Kumar and Khanna (2006) indicated that the application of *T. harzianum* in neem cake amended soil was highly effective against *M. incognita* and resulted in better plant status of tomato.

The carbofuran had very little residual effect on gall index, number of root-knot nematodes juveniles in the root and soil, and number of eggs compared with combined application fresh sweet orange and cassava peels and combined application of cassava and sweet orange peels. The higher population of root-knot nematodes in plots previously treated with carbofuran could be attributed to the exhaustion/degradation of carbofuran in soil in the third experiment. The time interval of the last application of carbofuran and planting of other experiment took five months. Smith *etal.* (2006) reported that carbofuran was found to be degraded in the soil at 90 days beyond which its effectiveness was significantly reduced.

In the case of the effect of organic amendments on the free-living nematodes, the fresh and dry cassava peels were found to increase the population of both bacterivorous and fungivorous nematodes more than the orange peels in the soil. Addition of organic amendments in the soil had resulted in population increase of free-living nematodes(Odeyemi *et al.*,2010; Thoden *etal.*, 2011).Jaffee (2006) reported that addition of organic amendments increased bacterivorous nematodes, but the nematode-parasitic fungus *Hirsutella rhossiliensis* decreased. Although, the cassava peel contain cyanide in the peels which kills nematode (Loumédjinon *et al*., 2007), but cyanide is easily degraded under high temperature and in water (Li and Chang, 2011). This low persistence of cyanide in cassava peels might have favoured the rapid multiplication of the bacterivorous and fungivorous nematodes in fresh and dry cassava peelstreatments.

For the experiment on the effect *T. viride* and organic amendment on plant growth factors, significant increases of the fresh shoot weight and shoot length of tomato were observed in the combined application of fresh sweet orange peel and *T. viride* more than the rest of treatements. This increment of the plant growth parameters in the combined application of fresh sweet orange peel and *T. viride* might be due to low population of the root-knot nematodes and galling and enhanced soil fertility condition. The level of damage caused by root-knot nematodes was positively correlated to the population present in the root and soil. The level of damage caused by root-knot nematodes has significant influence on the biomass and height of plant through competition in the limited nutrient resources in the soil (Corbett *etal*., 2011).

Furthermore, the highest fresh shoot weight and shoot length registered in the combined application of fresh orange peel and *T. viride* could be attributed to the supply of nutrients obtained from sweet orange peels. The result of the nutrient analysis in the organic amendments conducted at the Soil Science laboratory showed higher content of the macro-nutrients in the sweet orange peel than the cassava peel.

The number of root-knot nematode juveniles in the roots and soil, number of eggs, gall index and reproduction factor were significantly reduced in the combined application of fresh sweet orange peel and *T. viride* more than carbofuran. This significant reduction in the number of root-knot nematode and damage could be related to the combined effects of the fresh sweet orange peel and *T. viride.* Previous studies have shown greater potential in sweet orange peels in reducing pest population and damage on crops when applied in the soil. Karamaouna *etal*. (2013) indicated that essential oil of *C. sinensis* (mainly lemonene) was the most toxic of all the tested essential oils against crop pests. Andres *et al*. (2012) reported an increase in the rate of mortality of root-knot nematodes in the essential oil of *C. sinensis* with time after inoculation. In addition, *T.*

*harzianum*was observed to be an effective biocontrol agent for the management of plant-parasitic nematodes (Mukhtar *etal*., 2012). Similar result was observed by Dababat and Sikora (2007) who reported that *T. harzianum* and *T. viride* were found to significantly lower the number of root-knot nematode in the soil in the absence of tomato plants in the field. Therefore, considering the good potentials of the two treatments (fresh sweet orange peel and *T. viride*) in nematode management, their combination could have reduced the number of root-knot nematodes and damage more than the rest of the test treatments. Pedroche *etal*. (2009) reported that incorporation of broccoli residues and *T. harzianum* into soil significantly reduced root-nematode population and gall index in carrot compared with the single treatment.

The highest number of colonies of *T. viride* in the soil was observed in the combined application of fresh sweet orange peel and *T. viride*. This significant increase in the number of colonies under this treatment could be due to certain organic constituents in the fresh orange peels that help to enhance the development and reproduction of the fungus in the soil. Duli *etal*. (2013) reported that the media containing the extracts of peels from tangerine and sweet oranges were found to be the best among others in enhancing fungal growth and production of the spores.

4.10Conclusion and Recommendation

Application of sweet orange and cassava peels was effective in the management of root-knot nematodes on tomato. The laboratory results showed high percentage egg hatch inhibition and juvenile mortality after application of sweet orange and cassava peels.The fresh sweet orange peels had the highest percentage egg hatching inhibition and mortality of root-knot nematodes, followed by fresh cassava peels.The dry sweet orange peel was better than dry cassava peels with regards to percentage egg hatching inhibition and mortality of root-knot nematodes.

From the plant house experiment, root galling, number of root-knot nematode eggs and juveniles in the roots of tomato and soil were significantly reduced insoil amended with sweet orange and cassava peels. The application of poultry manure significantly increased the fresh shoot weight, root length and shoot length of tomato more than the rest of the treatments tested. The number of root-knot nematode juveniles in the root and soil, number of root-knot nematode eggs, root galling and reproduction factor were significantly reduced ($P<0.05$) in the application fresh sweet orange peels than all the other organic amendments. The fresh peels of cassava and sweet orange performed better in terms of reducing root-knot nematode population and damage than the dry peels of cassava and sweet orange.

For the field experiment, the combined application of fresh sweet orange and cassava peels significantly increased fresh shoot weight and plant height of tomato more than the other treatments tested. Also, the number of root-knot nematodes juveniles in the roots of tomato and soil, number of root-knot nematode eggs and root galling were highly reduced in the combined application of fresh sweet orange and cassava peels as good as carbofuran in the major and minor seasons. However, in observing the residual effect of various treatments, the plots previously treated with fresh sweet orange and cassava peels combination recorded reducednumbers of root-knot nematode juveniles in the roots of tomato and soil, number of eggs of root-knot nematodes and root galling than the rest of the treatments.The application of sweet orange peels increased the population of *T. viride*, a biocontrol fungus, while the cassava peels increased the population of bacterivorous and fungivorous nematodes in the soil. The plant growth, fruit size and yield of tomato were significantly increased in the combined application of fresh sweet orange and cassava peels than all the other treatments. The application of sweet orange peels (fresh or dry) highly increased soil nutrients such as organic

matter, total nitrogen, potassium and phosphorus than cassava peels. However, cassava peels increased the level of soil pH more than sweet orange peels.

In the plant house, the *T. viride* isolate obtained from the field significantly reduced the population of root-knot nematodes and root galling of tomato more than no application. However, the level of reduction in the number of root-knot nematodes in the roots of tomato and soil, number of eggs, root galling and reproduction factor were significantly higher in the combined application of fresh sweet orange peels and *T. viride* than all the other treatments tested. The application of fresh orange peels significantly increased the number of spore of *T. viride* than fresh cassava peels and carbofuran.

Therefore, combined application of FOP and FCP is recommended for adoption by farmers in Ashanti region through involvement of extension agents.Further evaluation is recommended on the effect of FOP and FCP combination on beneficial soil microorganisms. The FOP and FCP combination is recommended to complement integrated pest management approach. However, the use of dry peels (DOP and DCP) in different formulations (eg powder) is recommended if access to fresh peels is difficult

CHAPTER FIVE

5.0 EFFECT OF FRESH SWEET ORANGE AND CASSAVA PEELS ON THE ROOT-KNOT NEMATODES ISOLATED ON TOMATO FROM SEMI-DECIDUOUS FOREST AND FOREST SAVANNAH TRANSITION ZONES OF ASHANTI REGION, GHANA

5.1Introduction

Species of root-knot nematodes demonstrate a large diversity in various aspects of their life cycles.In most cases, the research on*Meloidogyne* spp. is frequently focusedon the three major species (*M. incognita, M. arenaria and M. javanica*) in the tropics.These species are mainly damaging to vegetables and cause losses up to 80% in heavily infested fields (Sikora and Fernandez, 2005). The ban on some synthetic nematicideshas prompted scientists to carry out research on environmentally-friendly and cost-effective alternative management methods (Rosskopf *et al*., 2005). The use of organic amendments for the management of root-knotnematodes has been

demonstrated in a large number of studies (Litterick *etal.*, 2004; Oka, 2010). Previous research result showed greater consistency in the use of different plant-based materials in the management of root-knot nematodes (Dias-Arieira*et al*., 2015). The application of these materials as soil amendment has great advantages in terms of biodegradability, environmental safety, renewability and management of target pests (Saravanapriya and Sivakumar, 2005).

The evaluation of the potential of sweet orange and cassava peels as an organic amendment in the management of root-knot nematodes gave positive results inprevious field experiments conducted at the research field at the Faculty of Agriculture.However, the root-knot nematode species present at this experimental site might be different from the ones in other parts of Ashanti Region. Therefore, further test on the efficacy of combined application of fresh sweet orange and cassava peels was done on the different root-knot nematode isolates collected from major tomato growing areas in Ashanti Region.

5.2Materials and Methods

5.2.1 Location of the Experiment

The experiment was carried out in the plant house, Faculty of Agriculture, Kwame

Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, from 28th June to 27th August, 2015.

5.2.2 Experimental Design

The experiment was arranged in Complete Randomized Design (CRD) with 19 treatments replicated four times. The root-knot nematode isolates collected from the root and soil of tomato plants in each area were treated with fresh sweet orange and cassava peel combination.

The root-knot nematode isolates were collected from the areas stated below:

1. Offinso Municipal (1*) isolates
2. Tanokrom isolates
3. Temati (1) isolates
4. Abolusu Magida isolates
5. Kumawu Kesekyi isolates
6. Dame Pataban isolates
7. Juaben isolates
8. Sabronum isolates
9. Temati (2) isolates
10. Bahankra isolates
11. Adutwie (Tuobodom) isolates
12. Akumadan (1) isolates
13. Akumadan (2) isolates
14. Nkenkensu isolates
15. Tanobuasi isolates
16. Offinso Municipal (2) strains
17. Control(treated)– Hortculture*
18. Control (untreated)- Horticulture*
19. Carbofuran – Horticulture*

***** The treatments served as control contained root-knot nematode isolates collected from horticulture, KNUST. The positive controls were separatelytreated with fresh sweet orange and cassava peels combination andcarbofuran. The no application served asnegative controls.The rest of the isolates from other parts of Ashanti Region were only treated with fresh sweet orange and cassava peels combination.

1* Numbers are allocated to isolates if more than one sample was collected in a given location.

5.2.3 Source, Preparation and Application of Fresh Sweet Orange and Cassava Peels

The sweet orange and cassava were the same variety and from the same source as described above. The peels were cut into small pieces of 0.5cm length and 0.5cm width. A rate of 25g of fresh orange peel was applied at four weeks before transplanting and 25g of fresh cassava peels applied at three weeks after transplanting.The fresh sweet orange peels were mixed with steam-sterilized topsoilriver sand mixture four weeks before transplanting. During application of the fresh cassava peels, about 300g of soil was removed from each pot and the cassava peels were evenly spread on the soil in the pot. The cassava peels were then covered with the removed soil. Carbofuran was applied at the rate of 0.5g per pot at 4 weeks before transplanting (Amulu and Adekunle, 2015).

5.2.4 Collection of Root and Soil Samples forExtraction of Root-knot Nematode Isolates

The isolates of *Meloidogyne* spp. were collected from six districts in the Semideciduous Forest and Forest Savannah Transition zones of Ashanti Region (Plate 5.1).The collection of samples was carried out when the tomato plants were at maturity and harvesting stages.The plants were uprooted at a depth of 0-15cm using hand trowel. The samples collected from each location were placed in plastic bags and properly labeled. The samples were taken to the Plant Pathology laboratory for extraction of root-knot nematode eggsand juveniles as described in experiment one.

Plate 5.1 Surveyed areas in the Semi-deciduous Forest and Forest Savannah Transition zones of Ashanti Region

5.2.4 Extraction and Counting of Root-knot Nematode Juveniles andEggsbefore Inoculation

The extraction and counting of the root-knot nematode juveniles and eggs were done as described at Section 4.5.1.12.

5.2.5 Sterilization of the Soil

The same process was used to sterilize the soil as described in experiment two. After sterilization, 1.8kg of topsoil-river sand mixture was placed in each of the 2 L plastic pots.

5.2.6 Inoculation of Root-knot Nematode Eggs

At four weeks before transplanting, the steam-sterilized top-soil in each pot was inoculated with root-knot nematode eggs extracted from samples from each location.

Two thousand five hundred eggs of root-knot nematodes were inoculated in each pot.

The preparation and application of fresh sweet orange and cassava peels were done as described experiment two.

5.2.7Preparation of the Nursery and Transplanting

Tomato variety "Power" was used as test crop. The same methodology for nursery preparation and transplanting was used as described in experiment two.

5.2.8 Extraction of Root-knot Nematode Eggs and Juveniles from the Rootand Soil after Harvest

At eight weeks after transplanting, the plants were uprooted and taken to the Nematology laboratory for extraction. The roots were gently washed with tap water to remove the soil and debris. The eggs were extracted from the roots using modified Hussey and Barker method, (1973). The juveniles were extracted from the soil using Baermann tray method (Whitehead and Hemming, 1965).

5.2.9 Data Collected

The following data were collected:

1) **Plant height (cm):** The plant height was measured at harvest. It was measured as described at section 4.5.1.16.
2) **Fresh shoot weight (g):** The fresh shoot of tomato plants were weighed after harvest. The same procedure was used as described at section 4.5.1.16.
3) **Fresh root weight (g):** The roots were weighed as described in experiment two.
4) **Gall index**:Galling was rated on the roots of tomato plants as described by Bridge and Page (1980).
5) **Number of eggs/5g root:** The eggs of root-knot nematodes were extracted from the roots of tomato and counted to determine the number per gramme of root. The same method was used as indicatedin section 4.5.1.16.
6) **Number of nematode juveniles/100g soil and 5g root:** The number of juveniles from the soil and roots of tomato were countedafter harvest. The procedure used was as described at section 4.5.1.16.

7) **Reproduction factor:** Reproduction factor was determined by dividing the final population of root-knot nematodes by the initial population (Pf/Pi).

5.2.10 Data Analysis

The data was subjected to Analysis of Variance (ANOVA) using GenStat 12th edition. The means were separated using Tukey"s test at P<0.05. The data on number of juveniles in the root and soil and number of eggs were normalized using $\sqrt{(x+1)}$transformation and back-transformed before statistical analysis.Where x is the mean counted.

5.3Results

5.4.1 Effect of Combined Application of Fresh Sweet Orange and Cassava Peels on Plant Growth Parametersof Tomatoin the Plant House

Table 5.1 shows the means ofthe fresh root weight/plant, fresh shoot weight/plant and shoot length of tomato tested under different treatments in the plant house. Significant differences (P<0.05) were observed between no application and the rest of the test treatments in terms of fresh root weight. The fresh root weight collected from carbofuran-treated pot was not significantly different(P>0.05) from all the other isolates treated with fresh sweet orange and cassava peels(Table 5.1).

The fresh shoot weight/plant significantly increased (P<0.05) under isolates treated with carbofuran and fresh sweet orange and cassava peels combined than no application (Table 5.1).However,carbofuran-treated pots were not significantly different (P>0.05) from all the other isolates treated with fresh orange and cassava peels combinedin terms of fresh shoot weight (Table 5.1).

Table 5.1 Effect of Combined Application of Fresh Sweet Orange and CassavaPeels on Fresh Root Weight, Fresh Shoot Weight and Shoot Length of Tomato Grown in the Plant House

Isolates/locations	Fresh root weight. (g)/plant	Fresh shoot weight. (g)/plant	Shoot length (cm)
Forest Savannah Transition			
Tanokrom isolate	3.1 b	21.5a	58.3 a
Temati isolate 1	3.6 b	22.5 a	57.4 a
Temati isolate 2	3.8 b	22.1 a	57.5 a
Aboluso Magida isolate	3.0 b	24.4a	61.7 a
Kumawu Kessenkyi isolate	3.1 b	23.6 a	59.8 a
Dame Pataban isolate	3.0 b	23.3 a	59.7 a
Bahankra isolate	3.0 b	24.9 a	59.8 a
Adutwie isolate	3.8 b	21.6a	57.7 a
Tanobuasi isolate	2.9 b	24.0 a	59.5 a
Semi deciduous Forest			
Offinso Municipal isolate 1	3.9 b	21.7a	55.5 a
Offinso Municipal isolate 2	3.8 b	22.2 a	57.4 a
Nkenkensu isolate	3.1 b	21.7a	58.9 a
Juaben isolate	3.2 b	21.9a	59.0 a
Akumadan isolate 1	3.5 b	22.1a	57.8 a
Akumadan isolate 2	3.5 b	21.5a	56.8 a
Sabronum isolate	2.7 b	23.1 a	55.27 a
Control			
Horticulturetreated isolate	3.0 b	22.3 a	60.4 a
Horticulture no applicationisolate	5.0 a	9.2 b	35.5 b
Horticulture – Carbofuran	2.8 b	22.2 a	56.7 a
LSD ($p<0.05$)	**1.2**	**2.9**	**5.6**
CV (%)	**13.5**	**9.5**	**6.9**

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$

The shoot length of tomato was significantly increased ($P<0.05$) in the combined application of fresh sweet orange and cassava peels and carbofuran-treated pots than no application. There were no significant differences ($P>0.05$) between the shoot length of tomato obtained in the application of carbofuran and pots treated with fresh sweet orange and cassava peels combination (Table 5.1).

5.3.2Effect of Combined Application of Fresh Sweet Orange and Cassava Peels on Gall Index, Number of Eggs, Number of Juveniles in the Rootsof Tomatoand Soil and Reproduction Factor of Root-knot Nematodes at the Plant House Gall index of the root-knot nematode isolates assessed under the different treatments at eight weeks after transplanting showed significant differences ($P<0.05$) among them (Table 5.2). Galling on the roots was significantly reduced ($P<0.05$) with the application of carbofuran and combined application of fresh sweet orange and cassava peels, compared to no application in horticulture isolates.No significant ($P>0.05$) differences were observed between carbofuran-treated pots and all the other pots treated with fresh sweet orange and cassava peels combination in terms of root galling.The number of root-knot nematode eggs were significantlyreduced ($P<0.05$) in the horticulture isolates treated with carbofuran and fresh sweet and cassava peel combination compared with the isolates in the no application (Table 5.2).

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Table 5.2 Effect of combined application of fresh sweet orange and cassava peelson the Population and Damage of Root-knot Nematode isolates of Tomato at the Plant House

Isolates/locations	[x]Gall Index	% Reduction of galling	[z]Eggs/5 g root	[z] Juveniles/5g root	[z] Nematodes/100 ml soil	[y] Reproduction factor
Forest Savannah Transition						
Tanokrom	3.0 b	58.9	1474 b	366 b	475b	0.3
Temati (1)	3.0 b	58.9	1446 b	353 b	488 b	0.3
Temati (2)	4.0 b	45.2	1433 b	352 b	463 b	0.3
Aboluso Magida	2.3 b	68.5	1255 b	295 b	413 b	0.3
Kumawu Kessenkyi	2.3 b	68.5	1389 b	331 b	450 b	0.3
Dame Pataban	2.5 b	65.8	1446 b	329 b	488 b	0.3
Bahankra	2.3 b	68.5	1324 b	313 b	425 b	0.3
Adutwie	3.8 b	47.9	1420b	336 b	475 b	0.3
Tanobuasi	2.3 b	68.5	1435 b	351 b	487 b	0.3
Semi deciduous Forest						
Offinso Municipal (1)	4.3 b	41.1	1479 b	373 b	470 b	0.3
Offinso Municipal (2)	3.8 b	47.9	1460 b	356 b	468 b	0.3
Nkenkensu	2.8 b	61.6	1384 b	329 b	488 b	0.3
Juaben	4.0 b	45.2	1385 b	316 b	463 b	0.3
Akumadan (1)	2.8 b	61.6	1340 b	325 b	450 b	0.3
Akumadan (2)	3.3 b	54.8	1353 b	326 b	488 b	0.3
Sabronum	4.0 b	45.2	1424 b	351 b	440 b	0.3
Control						
Control (treated)	2.5 b	65.8	1454 b	361 b	477 b	0.3
Control (no appli.)	7.3 a	-	3998 a	1457 a	2038 a	1.4
Carbofuran – Horticure	2.3 b	68.5	1252 b	289 b	400 b	0.3
LSD (p<0.05)	**2.0**	-	**262.1**	**104.5**	**84.4**	-
CV %	**11.8**	-	**6.1**	**7.4**	**7.5**	-

[z] Allnematode data were transformed, using $\sqrt{x}$, where x is the mean count before analysis and back-transformed

Means followed by different letter in the same column are significantly different, according to Tukey"s test at $P<0.05$

[x]Galling on scale 0-10 where 0 = no galls and 10 = root system completely galled (Bridge and Page, 1980) [y] Reproduction factor = Final population (Pf)/Initial population (Pi), where Pi = 2500

The number of root-knot nematode juveniles in the carbofuran-treated pots and combined application of fresh sweet orange and cassava peels were significantly

reduced (P<0.05) compared with horticulture isolates in theno application treatment (Table 5.2). However, there were no significant differences (P>0.05) between the application of carbofuran and combined application of fresh sweet orange and cassava peels in reducing the number of root-knot nematode juveniles in the roots of tomato (Table 5.2). Significant differences (P<0.05) were observed betweenthe number of juveniles of horticulture isolates in theno application control and the rest of the isolates treated with fresh sweet orange and cassava peels combination (Table 5.2).

The number of root-knot nematodes extracted from the soil were significantly reduced (P<0.05) in the application of carbofuran and combined application of fresh sweet orange and cassava peels than horticulture isolates in the no application (Table 5.2). However, the application of carbofuran was not significantly different (P>0.05) from the combined application of fresh sweet orange and cassava peels in reducing the number of root-knot nematode isolates extracted from the soil (Table 5.2).

Reproduction factor observed under the different test treatments varied from 0.3 to 1.4 nematodes (Table 5.2). The highest reproduction factor was recorded in the no application horticulture isolates (1.4), while thelowest (0.3) was observed in the isolates treated with carbofuran and combined application of fresh sweet orange and cassava peels (Table 5.2).

5.4Discussion

The fresh shoot weight and shoot length of tomato significantly increased in the combined application of fresh sweet orange and cassava peels than no application. The increase in the plant growth parameters in the combined application of fresh sweet and cassava peels might be attributed to improved soil condition and low infestation and damage of root-knot nematodes.The incorporation of cassava peels into the soil helped

to enhance fertility and improved the soilproperties with increase in plant growth and development (Okonkwo *et al*., 2011).

The combined application of fresh sweet orange and cassava peels was able to significantly reduce the population and damages of root-knot nematode isolates comparable to carbofuran. The significant reduction of root-knot nematodes could be due to the effectiveness of the nematicidal properties in fresh sweet orange(lemonene) and cassava (cyanide) peels. Lemonene insweet orange peels has been found to have nematicidal and nematostatic properties which enhanced egg hatching inhibition and mortality in*M.graminicola* (Steffen *etal*., 2008**).** Also,highermortality of root-knot nematodes andreduction in galling were observed in pepper after incorporation of cassava peels into the soil **(**Maina *etal*., 2012**).** Maňasová *etal*. (2012) reported total mortality (100%) of *Caenorhabditiselegans* after 270min of exposure to gaseous cyanide in the laboratory.

In addition, the effectiveness of the peels (fresh sweet orange and cassava)in suppressing root-knot nematode population and damage might also be due to the combined effect of the peels. This might have caused low reproduction factor of the isolates in the combined application of fresh sweet orange and cassava peels comparable to carbofuran. The first application of fresh sweet orange peels at four weeks before transplanting when the nematodes in the pot were already starved might have reduced the initial population significantly (Perry *et al*., 2009) and subsequent application of fresh cassava peels at three weeks after transplanting resulted in very low nematode population at harvest.Although, no research report has shown the combined effect of fresh sweet orange and cassava peels on root-knot nematodes, reports on the combined effect of other organic amendments have shown positive results. According to Agyarko *etal*. (2005), soils amended with neem leaf and cow dung combination had significantly

decreased the population of plant-parasitic nematodes in the soil than the single amendment.

5.6 Conclusion and Recommendation

The combined application of fresh sweet orange and cassava peels significantly increased fresh shoot weight and shoot length of tomato in all the pots than no application. Also, the number of root-knot nematodes, reproduction factor, galling of roots and number of eggs of all the root-knot nematode isolates tested on tomato were significantly reduced in the combined application of fresh sweet orange and cassava peels comparable to carbofuran. The combined application of fresh sweet orange and cassava peels reduced the population and damage of all root-knot nematode isolates collected from 19 different locations in the six districts of Ashanti Region. Further evaluation of the combined application of fresh sweet orange and cassava peels is recommended at farmers‟ fields where the root-knot nematode isolates were collected to confirm the result obtained from the plant house.

CHAPTER SIX

IDENTIFICATION OF ROOT-KNOT NEMATODE ISOLATES OF TOMATO FROMSEMI DECIDUOUS FOREST AND FOREST SAVANNAH TRANSITION ZONES USING MOLECULAR MARKERS

6.1 Introduction

Most of the root-knot nematodes(*Meloidogyne* species) are easily diagnosed by the presence of galls on the roots. Galls are formed as a consequence of physiological disturbances in the root tissues caused by the trophic interactions of female nematode.The identification of a particular root-knot nematode species is difficult, and typically requires taxonomic analysis, which is rarely feasible for most agriculturists

(Perry *et al*., 2009).There is no guarantee that an agricultural field contains only a single species of *Meloidogyne* or that the diagnostic descriptions currently available cover all of the diversity in the genus and will permit reliable identification(Karssen, 2005).

Root-knot nematode species are usually identified, using morphological features and morphometrics on second-stage juveniles (J2), males and on the perineal patterns of mature females or isozyme phenotyping of females (Hunt and Handoo, 2009). Morphometrics of juveniles can provide a relatively reliable assessment for species assignation but species-level identification, in practice, is complicated by genetic, climatic and anthropogenic factors associated with the dynamic nature and global scope of present-day agricultural production (Karssen, 2005).

Accurate identification of root-knot nematodes is crucial for effective disease control and depends on rapid and accurate classification of the pathogens involved so that appropriate control measures can be taken. Molecular biology techniques have the potential to increase the efficiency and sensitivity of this process. DNA analysis has been widely used in systematics and for identification of nematodes (Powers *et al*.,

2005). PCR assays have been used for identification of nematodes to species and are sensitive enough to identify the species of a single nematode (Powers *et al*., 2005).

Therefore, in order to confirm the presence of root-knot nematodes in the experimental sites, molecular analysis was done, using ITS-RLFP method.

6.2 Materials and Methods

6.2.1 Culturing of Root-knot Nematodes Species

Root-knot nematode isolates were collected from tomato in the 19 locations in two agro-ecological zones in Ashanti Region. Each sample was placed in a plastic bag and properly labelled. The eggs of the root-knot nematode isolates were extracted

separately,using Hussey and Barker method(1973). The extracted eggs of the rootknot nematode isolates from each location were inoculated in pots containing steamsterilized topsoil. Tomato var Power seedlings weretransplanted on the 15th June, 2015and allowed to grow with the nematode isolates for eight weeks. At the end of the eight weeks, the plants were uprooted and taken to the laboratory for purification, using single-egg mass.

6.2.2 Purification of the Root-knot Nematode Isolates

The roots of the infested-tomato plants were uprooted and taken to the Nematology laboratory, KNUST, for extraction of single-egg mass. The roots were gently washed underrunning tap water anddabbed dry with tissue paper. Each plant root wasplaced under stereo microscope and a total of five egg masses were separately removed, using a forcept. Each egg-mass was inoculated into a pot containing steam-sterilized topsoil and tomato var Power seedling. The root-knot nematode isolates were allowed to multiply for two months. One hundred grammes (100g) of soil was taken from each pot andfor root-knot nematode juveniles extractedusing Baermann tray method (Whitehead and Hemming, 1965).

6.2.3 Molecular Identification

The root-knot nematode isolates juveniles collected from the samples were identified, using ITS-RFLPs method(Powers *et al*., 2005).The Polymerase Chain Reaction (PCR)-RestrictionFragment LengthPolymorphisms (RFLPs**)** technique was used for molecular analysis.Pair of primers were used to amplify the Internal Transcribed Spacer Regions (ITS1 and ITS2) of nuclear ribosomal DNA. Amplified fragments were digested with 4 - 6bp restriction enzymes to detect variations.

The ITS-RFLPs was carried out according to the following procedures:

6.2.4 Extraction of DNA from Root-knot Nematodes

DNA of the root-knot nematode isolates was extracted from the 19 samples replicated five times using the Clear Detection extraction kit protocol (Subbotin *etal*., 2001). However, due to limited resources, only 80 samples out of 95 samples were used for DNA extraction. The working extraction buffer was formulated by mixing 6µl of Proteinase K, 15µl of 2-Mercapto-ethanol, 67.5µl of molecular bio-grade water and 75µl 2x extraction buffer, per sample. Fifty micro-liters (50µl) of each root-knot nematode isolate suspension was pipetted into extraction tubes containing 150µl of the extraction buffer. The suspension was incubated in the water bath at 65^{o}C for three hours and vortexed for one minute. The suspension was then transferred into new extraction tubes and a pellet was added to each tube. The suspension was vortexed for two minutes and then centrifuged at 1500g for one minute. The content was poured into the purification tubes on top of waste collection tubes and 350µl of the equilibration solution was added. The tubes were incubated at room temperature for five minutes and centrifuged again at 350g for one minute. The DNA purification tubes were then put on top of the DNA collection tubes and 50ul of each sample was added to the equilibrated DNA tube and incubated at room temperature for three minutes.The tubes were centrifuged again at 700g for one minute and the DNA was stored in the freezer at 4^{o}C for subsequent use.

6.2.5 Polymerase Chain Reaction (PCR) amplification

ITS-F (5'-TGTAGGTGAACCTGCTGCTGGATC-3') and ITS-R (5'-CCTATTTAGTTTCTTTTCCTCCGC-3') primers designed by Vrain *etal*. (1992)to amplify the ITS1 rDNA and ITS2 rDNA coding regions of *Meloidogyne* genomes wereused. The reaction mixture was prepared from 3.3µl sterile distilled water**,** 5.0µl of 2X deoxyribonucleotidetriphosphate(2X dNTPs),0.02µl of 25Mm $MgCl_2$, 0.3µl of 10Mm forward and reverse primers and 0.08µl of 15ug/l Taq polymerase per tube. One

microlitre (1µl) of each extracted DNA template was added to each tube to make a total reaction volume of 10µl per tube.Samples were spanned down and reactions were run in an Eppendorf Master Cycler machine (Techne TC-512 Thermal cycler, Germany) under the following thermal cycling conditions: initial denaturation at 94ºC for five minutes, 35 cycles of denaturation at 94ºC for four minutes, primer annealing at 54ºC for 30 seconds and 72°C for one minute extension, followed by a final extension at 72ºC for seven minutes. After the process, PCR products were brought out and kept in a freezer at 4ºC for further use.

6.2.6 Gel electrophoresis

Amplified PCR products were resolved on 2% agarose gel. The gel was prepared by mixing 2g of agarose with 100ml of 1X TAE buffer in a beaker. The solution was melted, using microwave oven for 10min until agarose totally dissolved in the buffer. The solution was allowed to cool and then 8µl of 0.01% ethidium bromide was added as staining solution to aid viewing under ultra violet light. The gel was poured into a tray with combs and allowed to set. The PCR products, 10µl each, were loaded into each well in an electrophoresis tank containing 1X TAE buffer and run at 120 volts for 2h. Seven micro-litres (7µl) of100 base pair DNA marker was used as ladder. The gel was removed after separation and viewed under a UV trans-illuminator. Photograph of gel was taken and sample DNA bands were scored as present (+) or absent (-), using the 100bp marker with respect to the expected amplicon size of760bp
(Qui *etal*., 2006).

6.2.7 The RFLP Method

The amplified PCR productswere analysed, using RFLP. The micro-tubes were properly labelled and arranged in the tube racks. The RFLPprocess was run, using the following formulation per sample: 12µl of sterilized distilled water, 2µl of 10X restriction enzyme

buffer, 2µl of restriction enzyme and 4µl of the respective DNA. Each reaction was carried out in a 200µl tube. The PCR product was digested, using the following restriction enzymes: Hinf I, Alu I, Taq I, Hind III and EcoR I (Metabion International AG, Germany). The content in the tube was mixed by centrifugation at 6,000rpm for three seconds and then incubated at 37°C for 6h. At the end of the incubation,0.2µl of 0.5 EDTA was added to each sample to stop the reaction. Samples were then loaded onto 2% agarose gel (containing 0.003% of ethidium bromide) and electrophoresed in 1X TAE buffer at 120 volts for 2h. Photographsof the bands were taken under ultra violet light.

6.3 Results

All the DNA samples analysed, using primer set, ITS-F (5'-TGTAGGTGAACCTGCTGCTGGATC-3') and ITS-R (5'-CCTATTTAGTTTCTTTTCCTCCGC-3') had the same amplicon size. Seventy-eight (78) out of the 80 samples amplified an expected 760bp amplicon (Plate 8.1), which confirmed preliminary results obtained, using morphorlogical identification. However, no amplification was observed in two samples (42 and 74) after resolution of amplified PCR products (Plate 8.1), which are found to be one of the replicates of Offinso Municipal isolate 2 and Tanobuasi isolate, respectively.

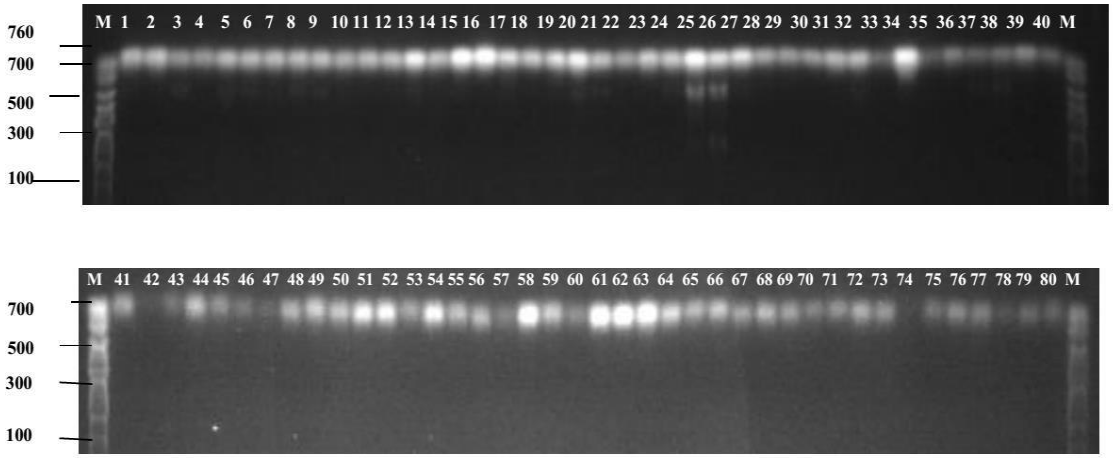

Plate 6.1 Gel showing Amplification of PCR Products

Key; M = DNA marker

Results of the RFLP analysis of the amplified PCR products with Hinf I, Alu I, Taq I, Hind III and EcoR I digests did not show clearly the presence of the different restriction band patterns. The bands were so highly smeared that it was impossible to distinguish the various nematode species. Therefore, the *Meloidogyne* spp. could not be identified to species level, using RFLP.

6.4Discussion

The ITS rDNA region of the genus *Meloidogyne* was amplified by using PCR technique. The PCR product showed that *Meloidogyne* spp observed in Ghana were all under the same ITS rDNA region. This result agrees with that ofQui *et al*. (2006) who found out the same sequences in the ITS rDNA regions for *M. incognita*, *M. arenaria* and *M. javanica.* However, the informatoin on the ITS rDNA region was insufficient to distinguish the species of *Meloidogyne* (Saeki *et al.*, 2014). Many reports have concluded that there is limited sequence polymorphism in the ITS sequences of the most common species such as *M. incognita*, *M. javanica* and *M. arenaria* (Perry *et al*., 2009). The amplicon size of the *Meloidogyne* spp collected was observed to be 760bp. Similar

result was observed by Naz *et al*. (2012) who reported that *Meloidogyne* spp collected from different locations showed the same ITS-restriction pattern with a band size of about 760bp. However, two of the samples did not amplify. This could be attributed to the low concentration of the DNA in those samples (Naz *et al*., 2012).

The RFLP analysis of the ITS rDNA region with the restriction enzymes was unable to show the presence of the different species. Although, it has been indicated that all the three most prevailent tropical species of *Meloidogyne* (*M. incognita*, *M. arenaria*, and *M. javanica*) can be distinguished by RFLP analysis targeting ribosomal or mitochondrial DNA (Harris *et al*. 2003), this was not the case in this study. The patterns were often not clearly seen against the background smear of DNA (Adam *etal*., 2007). However, previous research done in Ghana by Kwara *et al*. (2014) showed*M. incognita*, *M. arenaria*, and *M. javanica* as the common root-knot nematodes in vegetable in Ashanti Region with *M. incognita* being the most predominant.

6.5 Conclusion and recommendation

The ITS-RFLP molecular analysis method has confirmed the presence of *Meloidogyne* spp. in all the sampled areas. From the 80 DNA samples, only two were not amplified, which were Offinso Municipal 2 and Tanobuasi. The restriction enzymes did not digest any of the DNA samples. The study, therefore, recommends the use of single nematode to extract DNA instead of many nematodes. This will help to avoid contamination by other nematode species which are in mixed-community with *Meloidogyne* spp. in the soil. Also, the use of other simple and reliable method such as esterase method should be used for root-knot nematode identification.

CHAPTER SEVEN

DETERMINATION OF APPROPRIATE TIME OF APPLICATION OF SWEET ORANGE AND CASSAVA PEELS COMBINATION FOR ROOTKNOT NEMATODEMANAGEMENTON TOMATO IN THE PLANT HOUSE

KNUST

7.1Introduction

Organicamendments playacrucialroleinimprovingand maintaining soil quality. Besides their direct positive influence on soil fertility, soil structure and biology, they also affect the dynamics of soilborne pathogens, including plant-parasitic nematodes, by promoting antagonistic soil organisms, stimulating the competitive status of the non-pathogenic organisms, and by direct toxic effects during decomposition (Bonamoni *etal*., 2010). However, the effectiveness of organic amendments applied depends largely on the type, quantityapplied and time of application (Thoden *etal*., 2011).

The appropriate time of application of organic amendment is quite important as this could help to enhance the effectiveness of the product on the target pest and minimize the negative effect on plant growth. Ribeiro and Lima (2012) observedphytotoxicity on weeds after application of orange peel in the soil.From the laboratory experiment of chapter three, fresh orange peelreduced the population of root-knot nematode juveniles and suppressed egg hatching. However, there are reports from other researchers that the phytochemicals contained in orange peels were found to have allelopathic effect on plant growth. Therefore, in order to avoid negative side effects on crops and maximize nematicidal potentials, this experiment was designed to find out the appropriate time of application of fresh sweet orange and cassava peel combination.

7.2Materials and Methods

7.2.1 Experimental Design

The experiment was conducted in the plant house, Faculty of Agriculture, KNUST, from 1st March to 30th April, 2015. The experiment was arranged in a Completely Randomized Design (CRD) and replicated five times. The combined application of fresh sweet orange and cassava peels was used in this experiment because it performed better than the other treatments evaluated in the previous experiments. The following treatments were used:

1. Combined application of FOP + FCP* at 4 weeks before transplanting,
2. Combined application of FOP + FCP at 3 weeks before transplanting
3. Combined application of FOP + FCP at 2 weeks before transplanting
4. Combined application of FOP + FCP at 1 week before transplanting
5. Combined application of FOP + FCP at 4 weeks after transplanting
6. Combined application of FOP + FCP at 3 weeks after transplanting
7. Combined application of FOP + FCP at 2 weeks after transplanting
8. Combined application of FOP + FCP at 1 week after transplanting
9. Control (No application)
10. Carbofuran

* FOP=Fresh orange peels, FCP = Fresh cassava peels

7.2.2 Preparation of the Soil for Plant House Experiment

Thetopsoil-river sand mixture was preparedat 3:1 ratio (v/v). The preparation procedures were as described at Section 4.3.7.

7.2.3 Plastic Pots

Plastic pots,size 2 Lwere used. The pots were thoroughly washed with detergent and left to dry for a day before use.

7.2.4 Source of Seeds and Germination Test

The tomato variety “Power” was used as test crop. The seeds were obtained from the CSIR-CRI. Germination test of the seeds was conducted and germination was found to be 95%.

7.2.5 Root-knot Nematodes Source and Extraction

The eggs of root-knot nematodes were collected from infested tomato plants cultured in pots in the plant house. The plants were grown in steam-sterilized top-soil. The infested roots were collected and gently washed with tap water to reduce the soil on the roots. The eggs were extracted, using modified Hussey and Barker method (1973) as described in Section 4.3.7.

7.2.6 Type of Orange Peel Used

The fresh sweetorange peels were collected from sweet orange variety “Valencia”.
The peels were cut into small pieces (about 0.5cm length and 0.5cm width).
Preparation and application of the peels were done as described in experiment one.

7.2.7 Pre-experiment Treatment

About 5000 eggs of root-knot nematodes were thoroughly mixed with the steamsterilized soil mix. The pots were filled with steam-sterilized soil mix (1.7 L) and 21 day-old tomato seedlings were transplanted. The plants were watered daily for a period of eight weeks to facilitate egg hatching and enhance multiplication of rootknot nematode population. At the end of eight weeks, the plants were uprooted and soils in the pots were bulked together and mixed thoroughly. Also, juveniles in the roots were extracted and mixed into the bulked soil. In order to determine the population of root-knot nematode juveniles in the bulked soil, samples were randomly collected from different parts of the heap and mixed. A weight of one hundred grams
(100g) of the soil was sub-sampled to extract nematodes. The nematodes were extracted, usingBaermann tray method (Whitehead and Hemming, 1965). A total of 500root-knot

nematodes/100g soil was found as initial population before the implementation of the experiment.

7.2.8 Application of Amendmentsand Filling of the Pots

A quantity of 25g of fresh sweet orange peels and 25g of fresh cassava peels were mixed with 1.8 L of nematode-infested topsoil-river sand mixture. The soil mix was filled into 2 L pots. The treatments were applied as indicated at section 4.5.1.7.

7.2.9 Nursery Preparation and Transplanting of Seedlings

Tomato seeds were sown in steam-sterilized topsoil in wooden boxes. Watering and hardening of the seedlings were done as described in experiment two. Twenty-one day-old seedlings were transplanted in the pots. Seedlings of the same height were selected. One seedling was transplanted per pot.

7.2.10 Extraction of Eggs and Juveniles from Roots and Soil after Harvest The plants were uprooted at eight weeks after transplanting and root-knot nematode eggs and juveniles were extracted as indicated at section 4.3.8.

7.2.11 Counting of Root-knot Nematode Eggs and Juveniles

Counting was done by placing 1ml of the nematode-water suspension on the counting tray under the inverted compound microscope. The counting was done three times and the mean was calculated for each sample.

7.2.12 Data Collected

The following data were collected:

1. **Plant height (cm):** The heights of the plants were measured from the base of the stem to the tip of the tallest branch.
2. **Fresh shoot weight (g):**The fresh shoot weight of tomato was measured immediately after harvest.

3. **Fresh root weight (g):** The roots of tomato plants were cut at the base of the stem and weighed when they were fresh.
4. **Gall index:** The root galling was rated after harvest as described by Bridge and Page (1980).
5. **Number of nematodes/100g soil:** The number of root-knot nematodes juveniles extracted from the root and soil and number of eggs were counted after harvest. The methodusedwas as described at section 4.5.1.16.
6. **Number of root-knot nematode juveniles and eggs/5g root:** The number of juveniles and eggs of root-knot nematodes were counted as indicated at Section 4.5.1.16.
7. **Reproduction factor:** The reproduction factor was calculated by dividing the final population (Pf) by the initial population (Pi).

7.2.13 Data Analyses

The data were subjected to ANOVA, using GenStat statistical package 12th edition software. The means were compared, using Tukey"s test at $P<0.05$. All the root-knot nematode eggs and juvenile counts were square root transformed,using$\sqrt{(x+1)}$, where x is the mean of the nematodes counted before statistical analysis and backtransformed.

7.3Results

7.3.1 Effect of Time of Application of Fresh Sweet Orangeand Cassava Peels on Plant Growth factors of Tomato in the Plant House

Table 7.1 shows the effect of different times of application of fresh sweet orange and cassava peels combination on the shoot length, shoot weight and root weight of tomato. Theapplication of fresh sweet orangepeels at three weeks before transplanting significantly increased ($P<0.05$) the shoot length of tomato than the rest of the treatments in the plant house (Plate 7.1).However, no significant differences ($P>0.05$)

were observed between one, two, three and four weeks before transplanting in the shoot length of tomato. Significant differences (P<0.05) were observed between the combined application of fresh sweet orange and cassava peelsat three and four weeks before transplanting andthe application of carbofuran for the shoot length of tomato (Table 7.1).

The fresh shoot weight of tomato underdifferent application times of fresh orange and cassava peels combination showed significant differences (P<0.05)among the test treatments (Table 7.1).The combined application of fresh sweet orange and cassava peels at four weeks before transplanting significantly increased (P<0.05) fresh shoot weight of tomato (Plate 7.1), followed by three weeks before transplanting (Table 7.1). The fresh shoot weight in the combined application of fresh sweet orange and cassava peels at three and four weeks before transplanting weresignificantly different (P<0.05) from the rest of the treatments.

No significant difference (P>0.05) was observed between the application ofcarbofuran and the combined application of fresh sweet orange and cassava peels at one and two weeks before transplantingin terms of fresh shoot weight (Table 7.1).

Plate 7.1 Plant vigouras affected by the Applicationof Fresh Sweet Orange and

Cassava Peels at four Weeks before (A) and two weeks after Transplanting (B)

Table 7.1 Effect of Dates of Application of Fresh Sweet Orange Peel on Shoot Length, Fresh Shoot Weight and Fresh Root Weight of Tomato Plant atthe Plant House

Time of Treatment Application	Shoot length (cm)	Fresh shoot Wt.(g)/plant	Fresh root Wt. (g)/plant
4 weeks before transplanting	66.5 ab	27.2a	3.8 b
3 weeks before transplanting	69.3 a	26.9a	3.8 b
2 weeks before transplanting	65.6ab	19.9 b	3.8 b
1 weeks before transplanting	60.8 abc	18.1 b	3.4 b
4 weeks after transplanting	55.9 bc	10.1c	4.1ab
3 weeks after transplanting	52.2cd	9.8c	4.0 b
2 weeks after transplanting	40.7e	9.0 c	2.6 c
1 weeks after transplanting	41.7de	8.4 c	2.4 c
Carbofuran	56.5 bc	17.6 b	3.8 b
No application (control)	40.6e	7.1c	4.8a
LSD (p<0.05)	**10.8**	**3.7**	**0.7**
CV (%)	**6.9**	**7.2**	**5.7**

Means followed by different letters in the same column are significantly different, according to Tukey"s test at*P*<0.05

The fresh root weight/plant significantly increased (P<0.05) in the no application than the rest of the treatments (Table 7.1). Significant differences (P<0.05) were observed between no application and the rest of the application times, except thecombined application of fresh sweet orange and cassava peels at four weeks after transplanting. Also, combined application of fresh sweet orange and cassava peels at one and two weeks after transplanting were significantly different (P<0.05) from all the other treatments by recording the lowest fresh root weight per plant. No significant differences (P>0.05) were observed between the combined application of fresh sweet orange and cassava peelat one, two, three and four weeks before transplanting. Similarly, no significant differences (P>0.05) were observed between the application

times of fresh sweet orange and cassava peels combination at one and two weeks after transplanting for the fresh root weight (Table 7.1).

7.3.2 Effect of Different Application Dates of Fresh Sweet Orange Peelon Gall Index, Number of Eggs, Number of Juveniles in the Roots and Soil and Reproduction Factor of Root-knot Nematodes in the Plant House

The highest gall index was obtained in the no application control (7.2), whilst the lowest was observed in the combined application of fresh sweet orange and cassava peels at four weeks before transplanting (1.0) and Carbofuran (1.0) (Table 7.2). The combined application of fresh sweet orange and cassava peels at four weeks before transplanting significantly reduced ($P<0.05$) galling on tomato (86.1 %) compared to no application (Table 7.2).

The gall index recorded in the combined application of fresh sweet orange and cassava peels at one and two weeks before transplanting were significantly different ($P<0.05$) from the three and four weeks after transplantingtreatments (Plate 7.2). No significant differences ($P>0.05$) were observed between peels applied at one, two and three weeks before transplanting and one week after transplanting (Table 7.2).

Plate 7.2Severity of Galls as affected by the Application of Fresh Sweet Orange and Cassava Peels at four weeks before (A), no Application (B) and three weeks after Transplanting (C)

The number of eggs of root-knot nematodes was significantly reduced ($P<0.05$) in the application of carbofuran more than the rest of the treatments, followed by thecombined application of fresh sweet orange and cassava peels at four weeks before transplanting (Table 7.2).The number of eggs observed in the application of carbofuran and the combined application of fresh sweet orange and cassava peel at four weeks before transplanting were significantly lower($P<0.05$) than the rest of the treatments. However, no significant difference ($P>0.05$) was observed between carbofuran and the combinedapplication of fresh sweet orange and cassava peelat four weeks before transplanting in terms of number of eggs of root-knot nematodes(Table 7.2). The number of eggs of root-knot nematodes counted in the combined application of fresh sweet orange and cassava peels at three weeks before transplanting was significantly different ($P<0.05$) from the rest of the fresh sweet orange and cassava peels combination applied at weeks after transplanting (Table 7.2).

The application of carbofuran significantly ($P<0.05$) reduced the population of rootknot nematode juveniles in the roots morethan all the other treatments, followed by the combined application of fresh sweet orange and cassava peelat four weeks before transplanting (Table 7.2).Significant differences ($P<0.05$) were observedbetween the application of carbofuran and the rest of the application times except the combined application of fresh sweet orange and cassava peels at four weeks before transplanting (Table 7.2).Similarly, significant difference ($P<0.05$) was observedbetween the combined application of fresh sweet orange and cassava peelat three weeks before transplanting andthe rest of the peels applied at weeks after transplanting. However, the combined application of fresh sweet orange and cassava peel at one and two weeks

before transplanting were not significantly different ((P>0.05) from each other. Also, the number of root-knot nematode juveniles in thecombined application of fresh sweet orange and cassava peels at three and four weeks after transplanting were not significantly different (P>0.05) from each other (Table 7.2).

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Table 7.2 Effect of Application Dates of Fresh Sweet Orange and Cassava Peels onRoot Galling,Number of eggs, Number of Juveniles/5 g root and 100 ml soil and Reproduction Factor on Tomato in the Plant House

Time of Treatment application	[x] Gall index	% reduction of root galling	[z]No. of Eggs/5g root	No. of juveniles/5g root	No. of juveniles/ 100ml soil	[y] RF
4 weeks before transplanting	1 .0g	86.1	532 f	142 g	491 f	0.3
3 weeks before transplanting	1.3fg	81.9	636 f	173 f	655 e	0.4
2 weeks before transplanting	1.8 ef	75.0	1060 d	324 e	833 d	0.6
1 weeks before transplanting	1.8 ef	75.0	1133 d	334 e	872 d	0.6
4 Weeks after transplanting	6.2 b	13.9	2924 b	798 b	2094 b	1.4
3 Weeks after transplanting	5.0 c	30.6	2865 b	772 b	2011 b	1.4
2 Weeks after transplanting	3.5 d	51.4	2543 c	507 c	1078 c	0.8
1 Weeks after transplanting	2.3e	68.1	2421 c	459 d	1011c	0.7
Carbofuran	1.0 g	86.1	505 f	129 g	467 f	0.3
No application (control)	7.2 a	-	3430 a	1129 a	2844 a	2.0
LSD (p<0.05)	**1.1**	**-**	**132.9**	**31.9**	**136.2**	
CV (%)	**5.2**	**-**	**2.9**	**3.1**	**4.4**	

*Values with different letters in the same column are significantly different, according to Tukey"s test at$P<0.05$

[z]Nematode data were transformed using $\sqrt{}$ before analysisand back transformed. [y]Reproduction

Factor = final population (Pf)/Initial population (Pi), where Pi = 2000 eggs;

[x]Galling on scale 0-10 where 0 = no galls and 10 = root system completely galled;

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The number of root-knot nematodes extracted from the soil was significantly reduced ($P<0.05$) with the application of carbofuran and the combined application of fresh sweet orange and cassava peelat four weeks before transplanting more than the rest of the treatments (Table 7.2). Similarly, all the other application times of fresh sweet orange and cassava peel combination recorded significantly lower population of rootknot nematodes ($P<0.05$) from the no application (control) treatment. No significant difference ($P>0.05$) was observed between thecombined application of fresh sweet orange and cassava peelsat four weeks before transplanting and carbofuran for the number of root-knot nematodes extracted from the soil(Table 7.2).Also,the combined application of fresh sweet orange and cassava peelsat one and two weeks before transplanting were not significantly different ($P>0.05$) from each other. Similarly, no significant difference ($P>0.05$) occurred between peels applied at three and four weeks after transplanting (Table 7.2).

In the case ofreproduction factor of root-knot nematodes, the lowest was obtained in the application of carbofuran and the combined application of fresh sweet orange and cassava peels at four weeks before transplanting (0.3). The highest reproduction factor was recorded in the no application (2.0)(Table 7.2).

6.4Discussion

Thecombined application of fresh sweet orange and cassava peels before transplanting enhanced plant growth parameters and reduced number of eggs, number of juveniles extracted from root and soil and gall index of root-knot nematodeson tomato in the plant house. The application of fresh sweet orange at three and four weeks before transplanting significantly increased shoot length and fresh shoot weight of tomato more than the rest of the treatments. This can be attributed to the low number of rootknot nematodes and improved soil condition due to the combined application of organic

amendments at three to four weeks before transplanting. This duration (3 to 4 weeks) allowed for efficient and effective decomposition of the organic materials for eventual plant uptake.The nutrients in organic material can only be available to plant when they undergo the process of thorough decomposition (Okonkwo *etal*., 2011). Generally, the decomposition process follows a sequential pattern with different classes of organic compounds, which, in most cases,is affected bythe period (Habte *et al*., 2013).The optimum availability of nutrient in the soil was observed at 21 days after incorporation of the organic materials (Habte *et al,* 2013). Also, Mazzoncini*etal*. (2011)reported that an increase in soil NO_3-N was observed one month after incorporation of green manure.

The increased shoot length and fresh shoot weight in the combined application of fresh sweet orange and cassava peels at three and fourweeks before transplanting could alsobe related to the low population and gall index recorded. Anincrease in biomass and plant height of tomato was observed withreduction in the population of *M. javanica*in the field (Moslehi *etal*., 2010). The application of *Brassica* spp. plant parts into the soil significantly reduced the number of plant-parasitic nematodes in the soil and enhanced plant growth and development (Kim *etal*., 2011).

The time of application of the peels close to transplanting (oneto two weeks after transplanting) significantly reducedshoot length and fresh shoot weight. This might be due to phytotoxic effect ofthe orange peels around the root zone when the plants have not yet fully recovered from the transplanting shock.This result agrees with that of Ribeiro and Lima (2012) who indicated that orange peel was observed to have an inhibition effect on seedlings of *Euphorbiaheterophylla*by reducing the average size and malformation of roots. Similar result was obtained by Ibrahim *etal*. (2006) who reported thatlemonene was found to be phytotoxic to strawberries at concentrations beyond 3% and cabbage and carrot seedlings at concentrations exceeding 9%.

The root weight of tomato was significantly heavier inthe no application than all the other test treatments. In the no application pots, larger galls in terms of number and size were observed on the roots of tomato. Therefore, increase in root weight at the level of the control could be related tohigher number and larger size of galls on the roots of tomato at harvest.Serfoji *etal.* (2010) observed significantly higher root biomass in tomato plants with higher number andlarger sizesof galls in the glass house.Furthermore, significant increaseof gall index was observed as the time of the combined application of fresh sweet orange and cassava peels was delayed to four weeks after transplanting. The delay in application of the peels from two to four weeks after transplanting increased galling rate on tomato in the plant house. The increase in galling at four weeks after transplanting might be due to early infestation of the roots and in population of the root-knot nematodes immediately after transplanting. Thedelayin application of neem seed cake by three weeks after planting, enhanced galling and reduced the growth of lettuce(Aalders*etal*., 2009)

In the case of nematode parameters, the combined application of fresh sweet orangeand cassava peels at four weeks before transplanting performed as well ascarbofuran. The combined application of fresh orange and cassava peels at four weeks before transplanting significantly reduced the number of root-knot nematode juveniles in the root andsoil, number of eggs, gall index and reproduction factor of root-knot nematodes more than all the other treatments. According to Singh *etal*. (2008), application of neem cake at 25 days before planting of chickpea seeds significantly reduced *M. incognita* infestation more than the treatments applied after planting. This significant reduction in the application of fresh orange peel at four weeks before transplanting could be attributed to the action of the nematicidal propertiesat a timewhen there was inadequate food supply for the root-knot nematodes in the soil (Perry and Wesemael, 2008). It

could happen that the nematodes were only depending on the food reserved in their body,and, therefore, were highly susceptible to any chemical that might have negative effect on their physiological functions (Perry and Wesemael, 2008).In the absence of plants, nematodes become starved and vulnerable to nematicides (Lucas *etal*., 2012).

7.4Conclusion and Recommendations

The application of fresh sweet orange and cassava peel before transplanting reduced the effect of root-knot nematodes more effectively than after transplanting, but application at four weeks before transplanting was observed to be the most appropriate. The application of the peels at four weeks before transplanting significantly increased fresh shoot weight and shoot length of tomato more than all the other treatments.Also, the combined application of fresh sweet orange and cassava peels at four weeks before transplanting significantly reduced the number of root-knot nematodes in the soil, number of juveniles in the root, root galling and number ofeggs in the roots of tomato than the other treatments. The reproduction factor of the rootknot nematodes was also significantly reducedin combined application of fresh sweet orange and cassava peels at four weeks before transplanting, followed by three weeks before transplanting.

This experiment is recommended to be repeated in the field to validate the results obtained from the plant house experiment. Also, the experiment should be tried at different farmers‟ fieldsto encourage participatory research.

CHAPTER EIGHT

DETERMINATION OF MINIMUM EFFECTIVE DOSAGE OF FRESH SWEET ORANGE AND CASSAVA PEELS COMBINATION FOR ROOT-KNOT NEMATODE MANAGEMENT IN TOMATO IN THE FIELD

8.1Introduction

Several plant extracts are used to manage soil-borne pathogens such as plant-parasitic nematodes in vegetable production. Organic amendments have consistently been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and, thereby, improving the health of plants and reducing populations of plant parasitic nematodes (Mashela *et al*., 2008). A lot of research has been carried out on plant products such asneem seed cake and leaves, marigold and castor seed extracts(Oka, 2010; Adomako and Kwoseh, 2013)to help reduce population and damage of plant parasitic nematodes on crops. These plant products produce nematicidal (killing) and nematostatic (suppressive) organic compounds that are toxic to nematodes. The lemonene and cyanide related molecules extracted from orange and cassava peels, respectively, are natural pesticides which show greater potential for future use.Field and laboratory results on the effect of orange and cassava peels showed positive results.Karamaouna *etal*. (2013) reported that among the botanical extracts tested against mealybug on vines, the citrus peel essential oils (mainly lemonene) were found to be the most toxic of all the tested essential oils.The application of aqueous extracts of sweet orange (Tsai, 2008) and dry cassava peels as amendmentin the soil before plantingsignificantly reduced root-knot nematode population in carrot (Loumédjinon *etal*., 2007).

Olabiyi and Oladeji (2014) found out that 50g/kg soil withdry composted cassava peel was the most effective dose in reducing nematode population and enhance plant growth. However, the form of the peels, quantity to be applied and soil condition could significantly affect the effectiveness of the products on target organisms.The knowledge on the appropriate rateof fresh sweet orange and cassava peels combination against root-knot nematodes has not been studied.Therefore, the objective of this study was to

determine the appropriate rate of combined application of fresh sweet orange and cassava peels foreffective root-knot nematodes management on tomato in the field.

8.2Materials and Methods

8.2.1 Location of the Experiment

The experiment was conducted inthe research field of Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) from 23th July to 30th October, 2015.

8.2.2 Experimental Design

The treatments were laid in Randomized Complete Block Design with seven treatments replicated threetimes. Each experimental unit was allocated to a plot size of 2m x 4 m.The treatments were:

1. 30g offresh sweet orange peel + 30g of fresh Cassava peel per plant
2. 25g offresh sweet orange peel + 25g of fresh Cassava peel per plant
3. 20g offresh sweet orange peel + 20g of fresh Cassava peel per plant
4. 15g offresh sweet orange peel + 15g of fresh Cassava peel per plant
5. 10g offresh sweet orange peel + 10g of fresh Cassava peel per plant
6. No application (negative control)
7. 0.5g of carbofuran (positive control) per plant

8.2.3 Cropping History of the Experimental Site

Tomato had been grown at the site three months before the establishment of the experiment. The area was not burnt before application of the treatments. However, chemical fertilizer (NPK and urea) were applied on the previous crop.

8.2.4 LandPreparation andApplicationof Organic Amendments

The grasses on the field were cleared, using a cutlass. The soil was tilled and ridges were raised toabout 20cm high and90cm apart, using a spade. The peels were cut into small pieces (about 0.25cm length and 0.25cm width). The fresh sweet orange peels were applied at four weeks before transplanting, followed by fresh cassava peels at three weeks after transplanting. The method of application was as described in experiment three.

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8.2.5 Nursery Preparation and Transplanting of Seedlings

Tomato seedlings were grown on steam-sterilized topsoil in wooden boxes at the plant house. The seeds were sown in drills at a depth of 3cm and 10cm apart. At two weeks after emergence, the seedlings were hardened in the sun for two weeks before transplanting. Seedlings were transplanted at four weeks afternursery preparation. The seedlings were transplanted in the evening at a spacing of 50cm between plants and 90 cm between rows.

8.2.4 Fertilizer Application

NPK (15:15:15) and 46 % Nitrogen (urea) fertilizerswere applied at rates of 10 and 2g at fourand six weeks after transplanting, respectively.

8.2.5 Data Collected

A total of seven plants were randomly selected and tagged in each plot. All data collections were carried out on the seven plants that were tagged. The method of data collection was the same as described at section 4.6.2.

8.2.5.1 Initial Population of Root-knot Nematodes

A total of five core soil samples were collected from each plot and taken to the Nematology laboratory to determine the initial population of root-knot nematodes.
The method used was the same as indicated at section 4.6.2.1.

8.2.5.2 PlantHeight at Harvest (cm)

The heights of the plants were measured at harvest, using a measuring tape. The height was measured from the base of the plant to the tip of the growing point of the plant.

8.2.5.3 Weights of Shoot and Roots at Harvest

At harvest, three plants were randomly collected by uprooting from the net-plot (seven plants). The fresh shoot and roots were weighed separately to determine their weights.

8.2.5.4Number of Fruits and Yield (kg/ha)

The tomato fruits harvested from each of the seven plants were counted and weighed to determine the yield at maturity.

8.2.5.5 Determination of Gall Index of Root-knot Nematodes on Tomato Roots The roots were placed in a transparent glass bottle containing tap water. The roots were spread and galls were rated by comparing with diagram provided by Bridge and Page (1980) (Appendix 55).

8.2.5.6Number of Eggs and Nematodes in Roots and Soil at Harvest

At harvest, soil and root samples were collected at random from the central rows and used for extraction of root-knot nematodes. The counting of the eggs and nematodes were carried out using inverted compound microscope.

8.2.5.7Soil Analyses

Composite soil samples were systematically collected from the diagonalsof each experimental plot and sent to the Soil Science laboratory for analyses. The analyses were done on physical composition and pH. The methods used wereas described at section 4.6.2.8.

8.2.6 Data Analyses

The data were subjected to ANOVA, using GenStat. 12th edition software. The means were compared, using Tukey"s test at P<0.05. Nematode count data were square root transformed($\sqrt{(x+1)}$), where x is the mean count before analysis and back-transformed.Correlation analysis was done to find out the relationship between yield of tomato and root-nematode population.

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8.3Results

The soil was found to be sandy-loam with a composition of 65.57% sand, 9.03% clay and 25.40% silt. The pH of the soil was 7.11. The initial population of the root-knot nematodes before the application of the treatments was 612 nematodes/100g soil.The results of the effect of rates of combined application of fresh sweet orange and cassava peels on the growth factors of tomato and root-knot nematode data showed significant differences (P<0.05) between treatments.

8.3.1 Plant Height, Fresh Shoot Weight and Fresh Root Weight, Fruit Number, Fruit Sizeand Yield of Tomato under different Rates of Fresh Sweet Orange and Cassava Peelsin the Field

The effect of different rates of combined application of fresh sweet orange and cassava peels on plant height, fresh shoot weight and fresh root weight of tomato are shown in Table 8.1. The combined application of 30g of fresh sweet orange peel and 30g of fresh cassava peel had the highest plant height (91.4cm). The lowest plant height was observed in the no application (80.5cm). There wereno significant differences (P>0.05) between carbofuran and the different rates of fresh sweet orange and cassava peel combinations in terms of plant height (Table 8.1). However, all the different rates of the combined application of fresh sweet orange and cassava peels and carbofuran were significantly different (P<0.05) from no application with regard to plant height at harvest (Table 8.1).

Table 8.1 Effect of Different Rates of Fresh Sweet Orange and Cassava Peel Combination on Plant Growth and Yield of Tomato at Harvest (70 days after transplanting)

Treatments	Plant height at harvest (cm)	Fresh shoot weight (g)/plant	Fresh root weight (g)/plant	Number of fruits/plant	Fruit size (cm)	Yield kg/ha
30g FOP* + 30g FCP*	91.4 a	358.0 a	30.4 d	30 c	5.7 b	19697b
25g FOP + 25g FCP	90.9 a	355.8 a	32.0 cd	31 c	6.8a	20201a
20g FOP + 20g FCP	91.2 a	352.2 a	33.2 bc	31 c	5.8 b	19814b
15g FOP + 15g FCP	90.8 a	309.2 b	32.8 bc	32 c	4.9 c	18373c
10g FOP + 10g FCP	90.9 a	303.8 b	34.7 b	33 c	4.7 c	18027c
Carbofuran	87.6 a	295.4 b	31.9 cd	36 b	4.2 d	17384d
No application	80.5 b	125.2 c	43.9 a	41 a	3.2 e	8088e
LSD ($p<0.05$)	**5.9**	**18.3**	**1.9**	**2.9**	**0.4**	**386.7**
Cv (%)	**3.7**	**3.4**	**3.1**	**5**	**4.1**	**2.1**

*FOP= Fresh sweet orange peel

*FCP= Fresh Cassava peel

Means followed by different letters in the same column are significantly different, according to Tukey‟s test at $P<0.05$

The effect of different rates of the combined application of the peels on the fresh shoot weight/plant ranged from 125.2 to 358.0g (Table 8.1). The highest fresh shoot weight was observed in the application of 30g of fresh sweet orange and 30g of fresh cassava peels combination (358.0g). The lowest fresh shoot weight was recorded in the no application (125.2g). The fresh shoot weight of tomato in the application rates of 30g of fresh sweet orange and 30g of fresh cassava peels combination, 25g of fresh sweet orange and 25g of fresh cassava peels combination and 20g of fresh sweet orange and 20g of cassava peels combination were not significantly different ($P>0.05$) from each other.However,the fresh shoot weight of tomato was significantly greater ($P<0.05$) in the application rate of 25g of fresh sweet orange and 25g of fresh cassava peels combination than carbofuran (Table 8.1).

The fresh root weight/plant significantly increased ($P<0.05$) in the no application than the rest of the treatments. The combined application of 30g of fresh sweet orange peel and 30g of fresh cassava peel was significantly different ($P<0.05$) from the rest of the treatments except the combined application of 25g of fresh sweet orange peel and 25g of fresh cassava peel and carbofuran (Table 8.1). However, the combined application of 25g of fresh sweet orange peel and 25g of fresh cassava peel was not significantly different ($P>0.05$) from the combined application of 20g of fresh sweet orange peel and 20g of fresh cassava peel in terms of fresh root weight (Table 8.1).

The number of tomato fruits/plant was significantly higher ($P<0.05$) in the no application than the rest of the treatments tested (Table 8.1). Significant differences ($P<0.05$) occurred between no application and the rest of the treatments(Table 8.1). Similarly, significant differences ($P<0.05$) were observed between all the other rates of peels and carbofuran. However, no significant differences ($P>0.05$) were observed

between the different rates of combined application of fresh sweet orange and cassava peels in terms of number of fruits/plant(Table 8.1).

The fruit size of tomato ranged from 3.2 to 6.2 cm in diameter. The highest mean fruit size (6.2cm) was observed in the application rate of 25g of fresh sweet orange peel and 25g of fresh cassava peel combination, while the lowest fruit size was recorded in the no application (3.2cm). The fruit size of tomato significantly increased ($P<0.05$) in the applicationof 25g of fresh sweet orange peel and 25g of fresh cassava peel combination than the rest of the treatments (Table 8.1). Also, significant difference ($P<0.05$) was observed between the combined application of 20g of fresh sweet orange peel and 20g of fresh cassava peel and carbofuran. No significant difference ($P>0.05$) was observed between the application rate of 15g of fresh sweet orange peel and 15g of fresh cassava peel combination and the application rate of 10g of fresh sweet orange peel and 10g of fresh cassava peel combination (Table 8.1).Similarly, no significant difference ($P>0.05$) was observed between the applicationof 20g of fresh sweet orange peel and 20g of fresh cassava peel combination and the rate of 30g of fresh sweet orange peel and 30g of fresh cassava peel combination with regards to fruit size (Table 8.1).

The yield of tomato under the different rates of the combined application of the peels varied from 8088 to 20201kg/ha (Table 8.1). The application of 25g of fresh sweet orange peels and 25g of fresh cassava peels combination resulted in the highest yield (20201kg/ha). The lowest fruit yield was recorded in the no application (8088kg/ha). The rate of 25g of fresh sweet orange peel and 25g of fresh cassava peel combination was significantly different ($P<0.05$) from the rest of the treatments. However, application of30 and 20g of fresh sweet orange peel and 30 and 20g of fresh cassava peels combinations, respectively were not significantly different ($P>0.05$) from each other in terms of fruit yield of tomato (Table 8.1).

8.3.2 Effect of Rates of Fresh Sweet Orange and Cassava PeelsCombination on Gall Index, Number of Eggs and Number of Juveniles of Root-knot Nematodes Extracted from the Rootand Soil of Tomatoin the Field

The gall index of the different application rates varied from 1.3 to 6.7. The highest gall index due to root-knot nematode damage was significant ($P<0.05$) in the no application than the rest of the treatments (Table 8.2). Galling on the roots of tomato was significantly lower ($P<0.05$) in the combined application of 30, 25 and 20g of fresh sweet orange peels and 30, 25 and 20g of fresh cassava peels (80.6 %), respectively compared to the control (Plate 8.1). The gall index observed in all the other treatments were significantly different ($P<0.05$) from the no application. No significant differences ($P>0.05$) were observed between carbofuran and the combined application of 15 and 10g of fresh sweet orange peels and 15 and 10g of fresh cassava peels, respectively (Table 8.2).

Thenumber of eggs of root-knot nematodes ranged from 460 to 1232 eggs/10g root sample. The number of eggs extracted from the roots of tomato was significantly lower ($P<0.05$) in the combined application of 30, 25 and 20g of fresh sweet orange peel and 30, 25 and 20g of fresh cassava peel than the rest of the treatments except carbofuran (Table 8.2). However, there were no significant differences ($P>0.05$) between the combined applications of 30, 25 and 20g of fresh sweet orange peel and 30, 25 and 20g of fresh cassava peel, respectively. The number of eggs of root-knot nematodes observed in all the other treatments were significantly different ($P<0.05$) from the control. However, no significant difference ($P>0.05$) existed between the combined application of 15g of fresh sweet orange peel and 15g of fresh cassava peel and the combined application of 10g of fresh sweet orange peel and 10g of fresh cassava peel for number of eggs (Table 8.2).

Plate 8.1 Galling Severity as Affected by Application Rates of the Peels at 60g (A), 50g (B) and no Application

Table 8.2 Gall Index, Number of Eggs, Number of Juveniles of RootknotNematodes from the Rootsand Soil as affected by different Rates of Fresh Sweet Orange and Cassava Peels at the Field

Treatments	[x]Gall index	% reduction of root galling	[z]No. of eggs/10g root	[z]Juveniles/10 g root	[z]Juveniles /100ml soil
30g FOP*+30g FCP*	1.3 c	80.6	460 c	77d	183 d
25g FOP + 25g FCP	1.3 c	80.6	500 c	89 d	200 d
20g FOP + 20g FCP	1.3 c	80.6	524 c	90 d	233cd
15g FOP + 15g FCP	3.3 b	50.7	660 b	163c	283c
10g FOP + 10g FCP	3.7 b	44.8	724 b	215b	483b
Carbofuran	2.7 b	59.7	524 c	115d	250cd
No application	6.7 a	-	1232 a	313a	1050 a
LSD (p<0.05)	**1.1**	**-**	**84.3**	**49.6**	**109.5**
Cv (%)	**6.2**	**-**	**3.4**	**8.2**	8.5

Means followed by different letters in the same column are significantly different, according to Tukey"s test at $P<0.05$

[z]Data were transformed using $\sqrt{}$ and back-transformed before analysis [x]Galling on scale 0-10; where, 0 = no galls and 10 = root system completely galled

*FOP= Fresh sweet orange peel

*FCP= Fresh Cassava peel

The number of juveniles extracted from the root varied from 77 to 313 juveniles/10g root. The number of juveniles of root-knot nematodes in the roots of tomato were significantly reduced ($P<0.05$) in the combined application of 30, 25, 20g of fresh sweet orange peel and 30, 25, 20g of fresh cassava, respectively and carbofuran compared with the rest of the treatments(Table 8.2). However, no significant differences were obtained amongst these three treatments(Table 8.2). Also, carbofuran was significantly different ($P<0.05$) from the combined application of 15 and 10g of fresh sweet orange peel and 15 and 10g of fresh cassava peel, respectively. The number of juveniles of root-knot nematodes in all the other treatments were significantly lower ($P<0.05$) than no application (Table 8.2).

The effect of the rates of fresh sweet orange and cassava peels combination on the number of root-knot nematodes extracted from the soilis presented in Table 8.2. The highest number of root-nematode juveniles (1050 juveniles/100g soil) extracted from the soil was observed in the no application. The number of root-knot nematodes extracted from the soil was significantly lower($P<0.05$) in the application of 30, 25, 20g of fresh sweet orange peel and 30, 25, 20g of fresh cassava peel, respectively and carbofuran than the rest of the treatments (Table 8.2).However, the population extracted from the soil was not significantly different ($P>0.05$) between the combined application of 30, 25 and 20g of fresh sweet orange peels and 30, 25 and 20g of fresh cassava peels, respectively. Similarly, no significant difference ($P>0.05$) occurred between carbofuran and the combined application of 15g of fresh orange peel and 15g of fresh cassava peel (Table 8.2). There wassignificant difference ($P<0.05$) between the combined application of 15g of fresh sweet orange peel and 15g of fresh cassava peel and the combined application of 10g of fresh orange peel and 10g of fresh cassava peel. All the

treatments were significantly different (P<0.05) from the control with regardto number of root-knot nematodes from the soil (Table 8.2).

8.3.3 Relationship Between Root-Knot Nematode Population, Number of Eggs and Reproduction FactorandYield ofTomato.

The correlation analyses of the number of root-knot nematodes in the root of tomato and soil, number of eggs, gall index, reproduction factor and yield were highly significant (P<0.05) (Table 8.3). The gall index, number of root-knot nematodes extracted from the root and soil, number of root-knot nematode eggs and reproduction factor showed positive relationship between each other. However, there was negative relationship between the number of root-knot nematodes and yield of tomato (Table 8.3).

Table 8.3 Relationship betweenNumber of Root-knot Nematode Juveniles in theRoots and Soil, Number of Eggs, Root Gall, Reproduction Factor and Yield ofTomato

	No. of eggs/10g root	Gall index	No. nematodes/100g soil	of Reproduction factor	Yield kg/ha
Gall index	0.9042				
No. nematodes /100ml soil	0.9012	0.9141			
Reproduction factor	0.898	0.9262	0.977		
Yield kg/ha	-0.9092	-0.8741	-0.8963	-0.8836	
No. of Juveniles/10g root	0.7814	0.8451	0.8273	0.9024	-0.7101

8.3.4 Cost of production and Economic Return of the Organic Materials Used as Amendments in Tomato Production

Table 8.4 shows the cost of production and profit analysis of the different rates of fresh sweet orange and cassava peels evaluated in the field. The highest cost of production was recorded in the application of 30 g of fresh sweet orange and 30 g fresh cassava

peel combination (GH₵38154.2/ha), while the lowest was observed in no application with total production cost of GH₵ 35873.9/ha.However, highest economic return was observed in the application rates of 20 and 25 g/plant of fresh sweet orange and fresh cassava peels combination with benefit cost ration of 2.1 each.Anegative economic return was obtained in no application with GH₵ 0.90 in spending GH₵ 1.0 in the production of tomato (Table 8.4).

Table 8.4: Profit Analysis of the Rates of Organic Materials in Tomato Production at the Field. 2015 Major Season.

Treatments	Yield Mt/ha	Price/kg (GH₵)	TR/ha (GH₵)	TVC/ha (GH₵)	GP/ha (GH₵)	BCR (GH₵)
30g FOP+30g FCP	19697	4	78788	38154.2	40633.8	2.0
25g FOP + 25g FCP	20201	4	80804	37628.5	43175.5	2.1
20g FOP + 20g FCP	19814	4	79256	37093.3	42162.7	2.1
15g FOP + 15g FCP	18373	4	73492	36558.1	36933.9	2.0
10g FOP + 10g FCP	18027	4	72108	36022.9	36085.1	2.0
Carbofuran	17384	4	69536	36668.5	32867.5	1.9
No application	8088	4	32352	35873.9	-3521.9	0.9

TR =Total revenue, TVC= Total variable cost, GP= Gross profit, BCR = Benefit cost ratio

8.4Discussion

The plant growth parameters, number of root-knot nematodes in the roots and soil, number of egg and gall index were highly influenced by the application of fresh orange and cassava peels.The significant increase of fresh shoot weight, fruit diameter and yield of fruits in the above treatments could be attributed to the improved soil fertility andlow population and galling of root-knot nematodes on plants in those plots. Laboratory analyses of the nutrient contents showed that sweet orange and cassava peels contained major nutrients such as total nitrogen, potassium, available phosphorus, calcium and magnesium. The application ofcassava peel might have increased nutrient

composition of soil and enhanced growth and development of cabbage (Iren *etal*., 2015). In addition, the low number of root-knot nematode juveniles in the root and soil and gall index under the application of fresh orange and fresh cassava peels, respectively, might have caused the significant increase in fresh shoot weight, fruit size and yield of tomato. Olabiyi and Oladeji (2014) reported that application of dry cassava peels at 50g/kg of soil significantly reduced the number of nematodes in the soil and correspondingly increased fruit weight, number of fruits per plant and yield of okra over the control.According to Papachristos and Stamopoulos (2004), incorporation of the peels of *C. sinensis*at 30g/kg of soil was found to have fumigant toxicity, reproduction inhibitory effects, and repellent action against plantparasitic nematodes.

The yield of tomatosignificantly increasedin the application rate of 25g of fresh sweet orange peels and 25g of fresh cassava peels combination than the combined application of 30g of fresh sweet orange and 30g of fresh cassava peels. The increment in yield in the combined application of 25g of fresh orange and 25g of fresh cassava peels compared to the others could be due to large number of fruits and fruit size harvested (Luitel*et al*., 2012). The reduction in the number of fruits and size under the application of 30g of fresh sweet orange peel and 30g of fresh cassava peels might be attributed to phytotoxic effect due to application of orange peels beyond certain dose. Ibrahim *et al*. (2006) reported phytotoxicity in strawberries due to application of lemonene from orange peels at concentrations beyond 3%. The correlation analysis showed negative relationship between the number of root-knot nematode juveniles in the root and soil, number of eggs, root galling and reproduction factor and yield of tomato. Chareganiet*al*, (2012) observed significant reduction in the yield of tomato as the galling and initial population densities of both *M. javanica* and *M. incognita* increased.

For the number of root-knot nematodes extracted from the root and soil, number of eggs and gall index, highest reduction was observed in the application of 30, 25 and 20g of fresh orange and 30, 25 and 20g of fresh cassava peel, respectively.The effectiveness of these rates in reducing the nematode population, number of eggs and gall index of root-knot nematodes more than the other rates might be related to thehigh concentration of the phytochemicals in the peels that were lethal tothe nematodes and eggs. Nikoletta *etal*. (2013) reported that for orange peels to be effective in killing nematodes in the soil, certain dose has to be used. Also, Thoden *etal*. (2011) indicated that effectiveness of organic amendments applied depends largely on the type of organic amendment, quantity applied and time of application. The most active concentration of*C. sinensis* for root-knot nematodes control was found to be 2.0 mg/g of root (Nikoletta *etal*., 2013). In a similar research, Ntalli (2011) reported higher mortalityof *M. javanica* juveniles and lower galling in the application of peels of *C. sinensis* at a concentration of 0.01% (w/w) in cucumber. Also, Loumédjinon *etal.* (2007) reported that incorporation of 50g of dry orange peels and 50g of dry cassava peels in the soil were found to be effective in reducing nematode population in the root and soil, number of eggs and gall index in carrot. These wide differences with the rates of the current study result might be attributed to the higher concentration of phytochemicals found in the fresh orange and cassava peels than the dry ones. The laboratory analysis showed more than 50% reduction in cyanide content in fresh cassava peel as it was dried under shade for weeks in experiment two.

8.5Conclusion and Recommendation

The application of 20, 25 and 30gof fresh sweet orange and 20, 25 and 30 g fresh cassava peels combinations were found to be effective in reducing the population and damage of root-knot nematodes on tomato compared to the rest of the treatments. Highest fruit

size and yield of tomatowere obtained in the application rate of 25 g of fresh sweet orange and 25 g of fresh cassava peels but was not significantly different from the rate of 20g of fresh sweet orange and 20g of fresh cassava peels. The economic analysis showed equal return in profit in using the application rate of 20 and 25g of fresh sweet orange and 20 and 25g of fresh cassava peels as organic amendments in tomato production.Therefore, the application rate of 20 g of fresh sweet orange and 20 g of fresh cassava peels combination is recommended for adoption by farmers in Ashanti region. However, further evaluation of the rates is recommended in other regions of Ghana to validate the results.

CHAPTER NINE

GENERAL DISCUSSION

The presence of root-knot nematodes across different tomato growing areas poses a significant economic threat to the tomato industry. In Ashanti Region, although different types of pests and diseases prevail as constraints to vegetable production, there are many farmers engaged in tomato production. Attoh *et al*. (2014) indentified Ashanti Region as one of the areas with highest potential for tomato production in Ghana. Majority of tomato farmers in the study areas cultivated tomato crop on small scale. The dominance of small-scale farmers in the tomato production sector is one of the causes of low production in Ghana (Al-Hassan and Diao, 2006). Amongst the pests and diseases observed by farmers in tomato in the survey areas, root-knot nematodes were implicated as the most prevalent.The awareness creation of root-knot nematode infestation was carried out by the Ministry of Food and Agriculture (MoFA), Ghana where majority of the tomato farmers were trained. Dinham (2003) reported that majority of the vegetable farmers are aware of root-knot nematode damage on their

crops and indicated the pest as the most predominant in tomato. The high root-knot nematode infestation in tomato at farmers‟ fields might be attributed to the cultivation of susceptible tomato varieties. Majority of the tomato farmers in the survey areascultivated tomato variety “Power” which was found to be susceptible to root-knot nematodes (Kwara *et al*., 2014). Mitkowski and Abawi (2011) reported that higher number of root-knot nematodes were extracted from the roots and soil of susceptible carrot varieties than the resistant varieties.

Pesticides were found to be extensively used by the tomato farmers in the management of pests in tomato. Gerken *et al*. (2001) reported a rise in pesticide use over the years in Ghana mainly in the production of vegetables. This over-reliance of pesticide in the management of insect pests by tomato farmers in Ghana has also been reported by Okorley *et al*. (2002). None of the respondents used nematicides to control root-knot nematodes, however, all the respondents used crop rotation as control strategy against root-knot nematodes. Most of the farmers in the Ashanti Region practice tomato sole cropping (Anang *et al*., 2013), but rotate with pepper and cassava in every one to three years (Kyofa-Boamah *et al*., 2005).

The management of root-knot nematodes is becoming a great concern for vegetable producers and nematologists, because chemical nematicides are gradually disappearing (Collange *et al*., 2011). In the recent period, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides (Sorribas and Ornat, 2011). The application of these chemicals into the soil can have negative effects on non-target organisms as well as ground water pollution. According to Daramola *etal* (2013) indiscriminate and misuse of synthetic chemicals for pest control has led to problems such as pest resistance, adverse effect on non-target organisms and health hazards to the users.

Alternative techniques based on the use of organic amendments are needed to solve the problem. The essential oil from sweet orange peels exhibit biological activity against a wide spectrum of plant pests and may act as fumigants, contact insecticides, repellents, and antifeedants, or they can affect the growth rate, reproduction, and behavior of insect pests (Isman *et al*., 2008). Loumédjinon *et al.* (2007) reported that application of dry peels of sweet orange and cassava peels were found be as efficient as the commercial nematicide Rugby10 in reducing nematode population in the roots and soil of carrot. The present studies carried out at the laboratory, plant house and the field have shown potent nematicidal properties in sweet orange and cassava peels in the management of root-knot nematodes. Amongst the aqueous extracts of the organic amendments tested, fresh sweet orange peels had the highest egg hatching inhibition of root-knot nematodes, as compared to the rest of the treatments. Similar laboratory studies by Abolusoro *et al*. (2010) revealed that the highest concentration of dry sweet orange aqueous extract was able to inhibit egg hatching of *M. incognita* comparable to carbofuran. The egg hatch inhibition could be attributed to ability of the extract/compound to penetrate the gelatinous matrix and act on nematode eggs (Moreira *et al*., 2009). After their exposure to the fresh sweet orange aqueous extract, the hatch rate of egg masses immersed in water increased over time. Also, the percentage mortality was observed to be significantly higher in the aqueous extract of fresh sweet orange peels than all the other test treatments. This might be attributed to the presence of lemonene in the peels of sweet orange, which has been found to have higher nematicidal activity on both *M. javanica and M. incognita*compared to *Lavandula angustifolia*(Marino *et al*., 2012). The essential oil of *C. sinensis* was observed to cause 100% mortality at 72h of exposure (Kong *et al*., 2006). Generally, the fresh sweet orange and cassava peels were found to significantly inhibit egg hatching and increased mortality of root-knot nematodes more

than the dry peels. This could be due to higher concentration of nematicidal properties in the fresh peels, as compared to the dry peels. The concentrations of chemical compounds including limonene, were observed to be higher in the fresh peels of *C. sinensis* than the airdried peels (Adebisi, 2014).

For the plant house experiment, the fresh sweet orange and cassava peels were observed to increase the fresh shoot weight, shoot length and root length of tomato more than the other treatments tested. The increase in these agronomic parameters might be attributed to low numbers of root-knot nematodes in the root and soil as well as galling. Rizvi *et al*. (2014) attributed higher increase of biomass and shoot length of okra due to significant reduction in galling and number of root-knot nematodes in the root and soil in plots treated with neem seed cake.

Although the number of root-knot nematodes from the root and soil and gall index recorded in the application of fresh sweet orange and cassava peels were not significantly different from carbofuran, thus the increase in fresh shoot weight, shoot length and root length could be as a result of improved soil conditions from the application of the peels. Applications of organic amendments improved soil structure and enhanced growth and development of plants (Renco *et al.*, 2010). According to Mercy *et al*. (2014), peels of sweet orange and lime are rich in potash, zinc and iron and their incorporation into the soil enhanced growth of maize significantly than the no application. Similarly, Iren *et al*. (2015) indicated that application of cassavabased compost irrespective of the rate of application, enhanced the growth and yield of cabbage better than no application plots.

In addition, the application of fresh sweet orange peels significantly lowered the population of root-knot nematodes in the roots and soil and number of eggs comparable to carbofuran. This low population of root-knot nematodes and number of eggs in the

application of fresh sweet orange peels, compared to other peels, could be attributed to the presence of nematicidal properties. This observation agrees with that of Alam *et al.* (2005) who reported the effectiveness of lemonene in lowering the number of root-knot nematodesand enhancing egg hatch inhibition. The rate of galling due to root-knot nematode infestation was higher in the no application pots than the rest of the treatments. The high galling rate in the no application pot could be due to higher population of the root-knot nematodes. According to Kankam and Adomako (2014), increase in the number of root-knot nematodes in tomato resulted in corresponding increase in number of galls.

Furthermore, the same treatments were tried in the field to ascertain the consistency of their potentials in the management of root-knot nematodes. Interestingly, the peels of sweet orange and cassava that were combined, considering their effectiveness in the laboratory and plant house experiments, produced good results in the field in the major and minor seasons. The combined application of fresh sweet orange and cassava peels and combined application of fresh cassava and sweet orange peels significantly increased the fresh shoot weight and yield of tomato. The increase in fresh shoot weight and fruit yield in the combined application of fresh sweet orange and cassava peels and combined application of fresh cassava and sweet orange peels might be attributed to low number of root-knot nematodes. The nematode infestation acts as energy sink absorbing nutrients required by the plant for growth and fruit production, hence crop yields are reduced (Castagnone-Sereno, 2006). The incorporation of neem cake as organic amendment reduced the population of *M.incognita* and resulted in significantly increased fresh fruit yield and biomass of eggplant, compared with no application plots (Singh, 2013). Also, the higher fresh shoot weight and fruit yield could be caused by improved soil condition and higher presence of *T. viride* due the combined application

of the fresh peels. Organic amendments improve soil environment, benefit general plant health by helping with water retention and providing additional nutrients, and affect nematodes directly through producing detrimental products during decomposition (Krueger and McSorley, 2014). Singh (2013) indicated that combined application of neem seed cake and *P. fluorescens* and *T. harzianum* enhanced the microbial condition of the soil and increased the fruit yield of eggplant more than sole application of the treatments and no application plots.

For the effect of the organic amendments on the root-knot nematodes, the combined application of fresh sweet orange and cassava peels and combined application of fresh cassava and sweet orange peels performed equally well in terms of reducing number of root-knot nematodes in the root and soil, number of eggs and gall index in the major and minor seasons. This significant reduction of root-knot nematode population and damage with the combined application of fresh sweet orange and cassava peels and combined application of fresh cassava and sweet orange peels could be due to the effectiveness of the nematicidal constituents in the fresh sweet orange and cassava peels. Lemonene and cyanide are phytochemicals in sweet orange and cassava peels, respectively, that have been confirmed to be nematostatic and nematicidal (Andres *etal*., 2012; Ducom, 2012). Essential oil of *C. sinensis* is known for its ability to suppress plant-parasitic nematodes in the field (Krueger and McSorly, 2014) and further *in vitro* study revealed that different oil concentrations (1–4%) from *C. sinensis* had strong toxicity to eggs and juveniles of *M.incognita* (Adekunle *et al*., 2007). Ducom (2012) reported that hydrogen cyanide was found to cause total mortality of nematode larvae in woods after 18h of treatment.

In the second major season, where no application of the various treatments was made, the residual effects of the peels were observed on the plant growth factors and number

of root-knot nematodes extracted from the root and soil, gall index and number of egg on the roots. The residual effect of the combined application of fresh sweet orange and cassava peels increased fresh shoot weight, fruit size and fruit yield of tomato more than the rest of the treatments. The increase in fresh shoot weight and fruit yield in the combined application of fresh sweet orange and cassava peels might be attributed to low number of root-knot nematodes in the roots and soil and gall index.

The number of colonies of *T. viride* was higher in the application of fresh sweet orange and cassava peels than the rest of the treatment. Morton *et al*. (2004) reported that application of *Trichoderma* species, such as *T. harzianum, T. viride, T. atroviride* and *T. asperellum* have been found to reduce root-knot nematode population and galling,and increased plant growth and tolerance. Therefore, low root-knot nematode population, number of eggs and galling in plots treated with fresh sweet orange and cassava peels combination could be associated to the activity of *T. viride*.

Generally, the colony counts of the biocontrol agent, *T. viride,* was found to be higher in plots where fresh and dry sweet orange peels were applied, compared with cassava peels. The high number of colonies in the sweet orange peels could be attributed to certain phytochemicals present in sweet orange that must have enhanced the sporulation and multiplication the *T. viride* in the soil more than cassava peels. Ahmadi *et al.* (2013) found that *Trichoderma* spp. grew well on sweet orange peels than sugar beet pulp during fermentation of the crude protein.

The number of free-living nematodes and other fungi were observed to be greater in the sole application of fresh and dry cassava peels more than sole application of fresh and dry sweet orange peels and combined application of sweet orange and cassava peels. This might be attributed to fast degradation of hydrogen cyanide in the cassava peels which could have suppressed the population of the free-living nematodes in the soil.

Therefore, the organic by-product of the cassava peel might have been a good source of food for the organisms which enhanced their multiplication over the sweet orange peels (Dhas *et al*., 2011).

The test on the efficacy of indigenous *T. viride* and its combination with fresh sweet orange and cassava peels in the management of root-knot nematodes exhibited significant differences. The combined application of fresh sweet orange peels and *T. viride* increased fresh shoot weight and shoot length of tomato more than the rest of the treatments. This could be attributed to the low root-knot nematode population and damage in the combined application of fresh sweet orange peels and *T. viride* more than the other test treatments. Some *Trichoderma* isolates were reported to enhance plant growth and reduce root-knot nematode damage in tomato (Meyer *etal*., 2004). Khan *etal*. (2012) indicated that integration of *T. harzianum* and neem leaves resulted in significant increase in plant vigour and yield of eggplants and lower root-knot nematode damage.

For the effect of *T. viride* and organic amendments on the population and gall index of root-knot nematodes, the highest percentage reduction of root-knot nematodes from the roots and soil was observed in the combined application of fresh sweet orange peels and *T. viride*, compared with the other treatments. The high reduction in the population of root-knot at the level of combined application of fresh sweet orange peels and *T. viride* could be due to the combined effect of the peels and the biocontrol agent. *T. virids* has been found to be a potential biocontrol agent in the management of root-knot and other nematodes (Hallmann *et al*., 2009). Similarly, Abolusoro *et al*. (2010) reported that application of dry sweet orange peel in the soil reduced the number of *M. incognita* eggs and the second stage juveniles in the field due to the presence of saponins, flavonoids, and tannins. Therefore, the combined application of fresh sweet orange peels and *T.*

viride could have significantly reduced the, gall index, number eggs and number of root-knot nematode juveniles in the root and soil of tomato. Goswami *et al.* (2008) reported greater reduction in number of eggs and population of root-knot nematode juveniles in root and soil in the mustard cake and *T. viride*,combination compared with sole treatments.

In the case of the management of *Meloidogyne* spp. collected from different locations in Ashanti Region, the combined application of fresh sweet orange and cassava peels increased the fresh shoot weight and shoot length of tomato more than carbofuran at the plant house. This could be as a result of the application of fresh sweet orange and cassava peels which had improved the soil condition to enhance crop growth and development better than where carbofuran was applied.

Similarly, the application of fresh sweet orange and cassava peels significantly lowered the galling on the root, number of root-knot nematodes in the roots and soil and number of eggs as effective as carbofuran. The peels of sweet orange contain large amount of lemonene which is reported to be nematicidal (Andres *et al*., 2012). Also, the application of dry cassava peels was observed to reduce number of eggs and number of root-knot nematode juveniles in the root and soilof carrot (Loumédjinon *et al*., 2007), which is presumably due to the action of hydrogen cyanide (Ducom, 2012). Therefore, the combined application of the two peels (fresh sweet orange and cassava) could have had combined effect on the root-knot nematode population as much as carbofuran.

Further investigation was carried out on the appropriate time of application of fresh sweet orange and cassava peels. This was done to help avoid phytotoxicity and at the same time obtain maximum management of the root-knot nematodes on tomato. The combined application of fresh sweet orange and cassava peels at four (4) weeks before transplanting resulted in the best performance of tomato in the plant house. This might

be attributed to low population and damage of root-knot nematodes observed in the application of fresh sweet orange and cassava peels at four weeks before transplanting. Also, fresh sweet orange and cassava peel contained some nutrients such as nitrogen, phosphorus and potash. Therefore, the application of these peels could have been fully decomposed to release nutrients into the soil and increase fresh shoot weight and shoot length (Rizvi *et al*., 2012). The supply of nutrients such as potassium and silicon to plant during decomposition enhance plant resistance to pest and diseases through hardening of the vascular bundle (Ardakani, 2012). Oka *etal*. (2007) reported a reduction in the number of *Heterodera avenae* (Wollen) and *Meloidogyne marylandi* (Jepson and Golden) in wheat and oats after aerial application of potassium phosphite. This result is attributable to phosphite‟s ability to translocate along the plant‟s xylem and phloem (Wang and Bergeson, 2004).

On the effect of appropriate time of application of fresh sweet orange and cassava peels combination on the root-knot nematodes, the application at four weeks before transplanting gave the highest reduction of gall index, number of eggs and number of root-knot nematode juveniles in the root and soil. The higher reduction in root-knot nematode infestation and damage under this treatment could be due to the effect of the nematicidal properties in the peels when there was no food for the nematodes in the pots at that time (Oka and Yermiyahu, 2002).Limited amount of food available for the nematodes could have exacerbated the impact of the nematicidal properties compared to carbofuran. Abbasi *et al*. (2004) reported that application of neem seed cake at 28 days before planting of radish significantly reduced the population of plant-parasitic nematodes in soil and severity of damping-off compared with the rest of the application periods.

In the case of the effect of different rates of combined application of fresh sweet orange and cassava peels on plant growth parameters and root-knot nematode population, the highest fruit diameter and yield were obtained in the application of 25g of fresh sweet orange and 25g of fresh cassava peels combination. However, this rate was not significantly different from the application of 30g of fresh sweet orange and 30g of fresh cassava peel combination and 20g of fresh sweet orange and 20g fresh cassava peels combination. The increase in fruit size and yield under these application rates could be attributed to low number of root-knot nematodes and damage. The plant with root-knot nematode damaged roots showed decreased root system with fewer feeder roots (Anwar and Mckenry, 2010). Also, Anwar *et al.* (2009) reported that damage on root due to root-knot nematode infestation reduces the plant‟s ability to absorb available water and nutrients in the soil, which could result in poor vigour and yield loss.

For the number of root-knot nematodes extracted from the roots and soil and number of eggs, the highest reduction was observed in the application of 30g of fresh sweet orange and 30g of cassava peels combination, followed by 25g of fresh sweet orange and 25g fresh cassava peels combination and application of 20g of fresh sweet orange and 20g of fresh cassava peels, respectively. However, no significant differences occurred among them. The high reduction of the root-knot nematode population under the application rates mentioned above could be due to the level of concentration of the phytochemical present in peels that kill the nematodes. Perez *etal*. (2003) demonstrated that *C. sinensis* oil at 2, 4, 8, and 16µl/ml, significantly lowered egg hatching, juvenile survival and reproduction rate of root-knot nematodes. In a similar research, Koul *et al*. (2008) reported that 30g of sweet orange peel was found effective in reducing significant number of eggs and number of juveniles of root-knot nematodes in the screen house compared to control.

The investigation made on the gene regionusing ITS specific primers, showed the presence of *Meloidogyne* spp. in the surveyed areas. However, nematode DNA extracted from the samples tested from Offinso Municipal 2 and Tanobuasi did not amplify. This might be attributed to low concentration of the DNA available for analysis(Naz *et al*., 2012). The number of root-knot nematode juveniles extracted from these areas was small thus, the concentration of the DNA was low. All the PCR products could not be digested well with the restriction enzymes used. This could be due to the sensitivity of the RFLPs to smearing of the bands (Pulvirenti *et al*, 2005).Therefore, the *Meloidogyne* could not be identified to the species level. However, Kwara *et al*. (2014) used esterase method and reported the presence of*M. incognita, M. javanica and M. arenaria* in vegetable crops in Ashanti Region.

9.1 General Conclusionand Recommendation

The survey result shows that tomato farmers were faced with problems such as pests and diseases which limit the production of tomato in the study areas. Among the pest and disease problems stated, root-knot nematodes were found to be the most prevalent. The tomato farmers used various insecticides and fungicides to control insect pests and diseases on tomato. However, none of the respondents stated theuse of chemical nematicide to control root-knot nematodes on tomato. They used other management strategiessuch as crop rotation and weedingto control root-knot nematodes on tomato.

From the results of the efficacy trials,application of sweet orange and cassava peelswere effective in the management of root-knotnematodes on tomato. Percentage egg hatchinhibition and mortality occurred upon applicationof sweet orange and cassava peels. Also,galling, number of root-knot nematode eggs andjuveniles in the roots of tomato and soil werehighly reduced when soil was amended withsweet orange and

cassava peels. Application ofsweet orange peels enhanced the population of*T. virens*, a biocontrol fungus, while cassavapeels enhanced the population of bacterivorous and fungivorous nematodes.Also, damage and population of root-knot nematodes were reduced with corresponding increase in growth and yield of tomatoin plots previously treated with fresh sweet orange and fresh cassava peels combination compared with the rest of the treatments.

The combined application of fresh sweet orange and cassava peels was observed to be effective in reducing the population,number of eggs, gall index and reproduction factor of root-knot nematode isolates as well as carbofuran.

The timeof application of fresh sweet orange and cassava peels at four weeks before transplanting reduced damage and population of root-knot nematodes on tomato as effective as carbofuran. Also, the growth of tomato was significantly increased in the application of peels at four weeks before transplanting than all the other treatments except the application of peels at three weeks before transplanting.

For the minimum effective dosage of the peels, application of 20g of fresh sweet orange peels and 20g of fresh cassava peels was found to be as economically viable as the application of 25g of fresh sweet orange peels and 25g of fresh cassava peels combination.

The molecular analysis confirmed the presence of *Meloidogyne*spp. in all the 19 samples collected from Ashanti Region. However, two of the DNA samples, Offinso Municipal isolate 2 and Tanobuasi isolate did not amplify due to low concentration of the DNA.

Therefore, the study recommends the following:

1. Tomato farmers should be sensitized by extension agents on the use of sweet orange and cassava peels in the management of root-knot nematodes on tomato
2. The combined application of fresh sweet orange and cassava peelsto be released for adoption by farmers in Ashanti region
3. Further evaluation is recommended on the determination of appropriate time of application of fresh sweet orange and cassava peels at the field.
4. Minimum dosage of 20 g of fresh sweet orange and 20 g of fresh cassava peels combination is recommended for adoption by tomato farmers in Ashanti region
5. Fresh sweet orange and cassava peels is recommended to complement Integrated Pest Management (IPM) through the application of the peels and planting of tomato varieties tolerant to root-knot nematodes.
6. The use of single root-knot nematode for DNA extraction is recommended to avoid contamination.
7. In addition to ITS-RFLP method, other more reliable methods such as isozymes should be tried to identify the species of *Meloidogyne*.

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APPENDICES

Appendix 1: Questionnaire administered in the farmers' perception survey

A SURVEY ON FARMERS"PERCEPTION

Please be informed that information collected from this survey will be used only for the write-up of the PhD thesis.

KNUST

Enumerator Data sheet no Date……..

Zone District Village……

Respondent ……..

Ageyears Sex: MFEthnicity……..

1) Do you own this land? A) Yes B) No

2) How long have been cultivating the land?

A) 2 years. B) 3 years. C) 4 years D) 5 years. E) More than 5 years F) others (specify)

3) What types of vegetable crops do you grow?

Vegetable **Variety** **Area** **Priority** i) ii)

iii)

iv)

1) How many times do you grow tomato in a year?
2) What is ones best time to plant? Why?
3) What types of crops are you currently growing?
4) What is the stage of your crop?
5) Do you practice crop rotation? A) Yes B) No

If yes, what types of vegetables have been growing on your piece of land during the past 3 years?

6) What is the sequence of crop?

7) What are your reasons for the choice of crop and cropping sequence?

11) Do you apply fertilizers to your vegetables? A) Yes B) No 12)

What type of fertilizers do you apply? A) Urea B) NPK C) Manure 13)

How much quantity of fertilizers do you apply in Kg/ha?

14) Are you aware of nematodes as vegetable pests? A) Yes B) No

15) Do you encounter any pest damage on your vegetable crops? A) Yes B) No

If yes, what types of pests do cause damage?

16) Do you attempt to control the pests? A) Yes B) No

If yes, what type/types of control measures do you practice? A) Insecticides B) Botanicals C) Fungicides D) nematicides E) Others (specify).

17) How do you apply the pesticides ?

18) How much do you apply for a hectare?

Appendix 2: Effect of organic amendments on egg inhibition of root-knot nematodes at the laboratory.

Analysis of variance

Variate: Percent egg inhibition

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	6	72984.65	12164.11	770.81	<.001
Observ_days	2	14895.83	7447.91	471.96	<.001
Treatments.Observ_days	12	6692.44	557.70	35.34	<.001
Residual	84	1325.60	15.78		
Total	104	95898.51			

LSD: 4.996

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Appendix 3 Effect of organic amendments on mortality of root-knot nematodes at the laboratory.

Analysis of variance

Variate: Percent nematode mortality

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	4	53685.253	13421.313	7743.07	<.001
Observ_Time	2	19972.160	9986.080	5761.20	<.001
Treatments.Observ_Time	8	5290.907	661.363	381.56	<.001
Residual	60	104.000	1.733		
Total	74	79052.320			

LSD: 1.7

Appendix 4: Plant height of tomato as affected by organic amendments at the plant house.

Analysis of variance

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	1657.83	236.83	16.16	<.001
Residual	32	468.95	14.65		
Total	39	2126.78			

LSD: 4.9

Appendix 5 Root length of tomato as affected by organic amendments at the plant house.

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Analysis of variance

Variate: Root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	45.5360	6.5051	7.85	<.001
Residual	32	26.5200	0.8287		
Total	39	72.0560			

LSD: 1.1

Appendix 6: Fresh shoot weight of tomato as affected by organic amendments at the plant house.

Analysis of variance

Variate: Shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	104.668	14.953	14.93	<.001
Residual	32	32.056	1.002		
Total	39	136.724			

LSD: 1.3

Appendix 7 Fresh root weight of tomato as affected by organic amendments at the plant house.

Analysis of variance

Variate: Fresh root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

:

Treatments	7	6.2147	0.8878	3.67	0.005
Residual	32	7.7459	0.2421		
Total	39	13.9605			

LSD: 0.63

Appendix 8: Effect of organic amendments on the number of root-knot nematodes in the soil at the plant house.

Analysis of variance

Variate: Number of root-knot nematodes/100ml soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	15178681.	2168383.	440.26	<.001
Residual	32	157607.	4925.		
Total	39	15336288.			

LSD: 1.19

Appendix 9 Effect of organic amendments on the number of root-knot nematode juveniles in the root of tomato at the plant house.

Analysis of variance

Variate: Number of root-knot juveniles/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	2362.8756	337.5537	948.49	<.001
Residual	32	11.3883	0.3559		
Total	39	2374.2640			

:

LSD: 0.77

Appendix 10: Effect of organic amendments on the number of root-knot nematode eggs in the root of tomato at the plant house.

Analysis of variance

Variate: Number of egg/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	4918.120	702.589	166.23	<.001
Residual	32	135.255	4.227		
Total	39	5053.375			

LSD: 2.65

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Appendix 11 Effect of organic amendments on the root-knot nematode gall index at the plant house.

Analysis of variance

Variate: Gall Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	305.3750	43.6250	183.68	<.001
Residual	32	7.6000	0.2375		
Total	39	312.9750			

LSD: 0.63

Appendix 12: Effect of organic amendments on the reproduction factor of rootknot nematodes at the plant house.

Analysis of variance

Variate: Reproduction factor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	8.917864	1.273981	1009.20	<.001
Residual	32	0.040396	0.001262		
Total	39	8.958259			

LSD: 0.05

Appendix 13 Effect of sweet orange and cassava peels on the plant height of tomato on the field Analysis of variance

Variate: Plant height at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	1645.29	235.04	15.99	<.001

:

Residual	32	470.47	14.70
Total	39	2115.76	

LSD: 4.94

Appendix 14: Fresh shoot weight of tomato as affected by the application of sweet orange and cassava peels on the field

Analysis of variance

Variate: Fresh shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	108.403	15.486	14.15	<.001
Residual	32	35.016	1.094		
Total	39	143.419			

LSD:1.35

Appendix 15 Fresh root weight of tomato as affected by the application of sweet orange and cassava peels on the field

Analysis of variance

Variate: Fresh root weight at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	6.6479	0.9497	2.66	0.028
Residual	32	11.4314	0.3572		
Total	39	18.0794			

LSD: 0.77

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Appendix 16: Number of root-knot nematode juveniles in the roots as affected by the application of sweet orange and cassava peels on the field

Variate: Number of Juvenile/10g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	196669.	65556.	8.45	
REP.*Units* stratum					
TRT_1	10	9321483.	932148.	120.16	<.001
Seasons	1	4974.	4974.	0.64	0.426
TRT_1.Seasons	10	561048.	56105.	7.23	<.001
Residual	63	488745.	7758.		
Total	87	10572920.			

LSD: 124.5

Appendix 17 Effect of sweet orange and cassava peels on the galling of rootknot nematodes on the field

Variate: Gall_index (Season 1 and 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.5189	0.1730	0.97	
REP.*Units* stratum					
TRT_1	10	278.3737	27.8374	155.38	<.001
Seasons	1	0.0013	0.0013	0.01	0.933
TRT_1.Seasons	10	13.7071	1.3707	7.65	<.001
Residual	63	11.2866	0.1792		

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Total	87	303.8876			

LSD: 0.6

Appendix 18: Effect of sweet orange and cassava peels on the number of rootknot nematodes in the soil on the field

Analysis of variance

Variate: Number of Nematode/100g soil (Season 1 and 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	85531.	28510.	3.45	
REP.*Units* stratum					
TRT_1	10	56683105.	5668310.	685.22	<.001
Seasons	1	79382.	79382.	9.60	0.003
TRT_1.Seasons	10	4762632.	476263.	57.57	<.001
Residual	63	521150.	8272.		
Total	87	62131800.			

LSD: 128.5

Appendix 19 Number of root-knot nematode eggs as affected by the application sweet orange and cassava peels on the field

Variate: Number of eggs/10g root (Season 1 and 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	3278275.	1092758.	8.20	
REP.*Units* stratum					
TRT_1	10	62816268.	6281627.	47.16	<.001
Seasons	1	75263.	75263.	0.57	0.455
TRT_1.Seasons	10	10049497.	1004950.	7.54	<.001
Residual	63	8391666.	133201.		

:

Total	87	84610969.

LSD: 515.7

Appendix 20: Fruit yield of tomato as affected by the application sweet orange and cassava peels on the field

Variate: Yield per hectare (Season 1 and 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT_1	10	1.176	1.176	469.64	<.001
Seasons	1	4.006	4.006	1599.37	<.001
TRT_1.Seasons	10	1.534	1.534	6.12	<.001
Residual	66	1.653	2.505		
Total	87	1.609			

LSD: 706.6

Appendix 21 Effect of organic amendments and *T. viride* on the fresh root weight of tomato in the plant house

Variate: Fresh root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	11.52240	2.88060	73.11	<.001
Residual	20	0.78800	0.03940		
Total	24	12.31040			

LSD: 0.79

Appendix 22: Effect of organic amendments and *T. viride* on the shoot length of tomato in the plant house.

Analysis of variance

Variate: Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

:

TREATMENT	4	1652.090	413.023	236.45	<.001
Residual	20	34.936	1.747		
Total	24	1687.026			

LSD: 1.74

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Appendix 23 Effect of organic amendments and *T. viride* on the fresh shoot weight of tomato in the plant house.

Analysis of variance

Variate: Fresh shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	461.8056	115.4514	194.10	<.001
Residual	20	11.8960	0.5948		
Total	24	473.7016			

LSD: 1.0

Appendix 24: Effect of organic amendments and *T. viride* on the galling of rootknot nematodes in tomato in the plant house.

Variate: Gall index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	132.5600	33.1400	92.06	<.001
Residual	20	7.2000	0.3600		
Total	24	139.7600			

LSD: 0.79

:

Appendix 25 Effect of organic amendments and *T. viride* on the number of rootknot nematodes eggs on tomato in the plant house.

Variate: Number of eggs/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	25510975.	6377744.	623.98	<.001
Residual	20	204420.	10221.		
Total	24	25715395.			

LSD: 133.4

Appendix 26: Number of root-knot nematode juveniles in the roots as affected by the application organic amendments and *T. viride*in the plant house.

Variate: Number of root-knot nematode juveniles/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	3358592.	839648.	87.18	<.001
Residual	20	192631.	9632.		
Total	24	3551223.			

LSD: 129.5

Appendix 27: Number of root-knot nematode juveniles in the soil as affected by the application of organic amendments and *Trichoderma viride*in the plant house.

Variate: Number of root-knot nematodes/100ml soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	14901400.	3725350.	376.30	<.001
Residual	20	198000.	9900.		
Total	24	15099400.			

:

LSD: 131.3

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Appendix 28: Reproduction factor of root-knot nematodes as affected by the application organic amendments and*T. viride* in the plant house.

Variate: Reproduction factor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	4	8.001218	2.000304	301.73	<.001
Residual	20	0.132590	0.006630		
Total	24	8.133808			

LSD: 0.11

Appendix 29: Effect of sweet orange and cassava peels combinedon fresh root weight of tomato at the plant house.

Variate: Fresh root Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	29.3138	1.6285	5.95	<.001
Residual	57	15.5985	0.2737		
Total	75	44.9123			

LSD: 0.74

Appendix 30 Effect of sweet orange and cassava peels combinedon fresh shoot weight of tomato at the plant house.

Variate: Fresh shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	1877.32	104.30	6.74	<.001

:

Residual	57	882.29	15.48	
Total	75	2759.60		

LSD: 4.52

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Appendix 31: Effect of sweet orange and cassava peels combinedon fresh shoot length of tomato at the plant house.

Analysis of variance

Variate: Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	4614.98	256.39	7.50	<.001
Residual	57	1948.06	34.18		
Total	75	6563.04			

LSD: 8.2

Appendix 32 Effect of sweet orange and cassava peels combinedon number of root-knot nematode eggs from tomato roots at the plant house.

Analysis of variance

Variate: Number of eggs/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	42999330.	2388852.	23.02	<.001

:

Residual	57	5915799.	103786.		
Total	75	48915128.			

LSD: 456.2

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Appendix 33: Effect of sweet orange and cassava peels combinedon number of root-knot nematode juveniles extracted from roots of tomato at the plant house.

Analysis of variance

Variate: Number of root-knot nematode juveniles/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	6305002.	350278.	51.24	<.001
Residual	57	389677.	6836.		
Total	75	6694679.			

LSD: 117.1

Appendix 34 Effect of sweet orange and cassava peels combinedon number of root-knot nematode juveniles extracted from soil at the plant house.

Analysis of variance

Variate: Number of root-knot nematodes/100ml soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	10327829.	573768.	50.03	<.001
Residual	57	653750.	11469.		

:

Total	75	10981579.

LSD: 151.6

Appendix 35: Effect of sweet orange and cassava peels combinedon reproduction factor of root-knot nematodes on tomato at the plant house.

Analysis of variance

Variate: Reproduction factor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	5.209440	0.289413	63.78	<.001
Residual	57	0.258654	0.004538		
Total	75	5.468095			

LSD: 0.10

Appendix 36 Effect of sweet orange and cassava peels combinedon galling of root-knot nematodes on tomato at the plant house.

Analysis of variance

Variate: Gall Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Isolates	18	153.7895	8.5439	15.71	<.001
Residual	57	31.0000	0.5439		
Total	75	184.7895			

:

LSD: 1.04

Appendix 37: Effect of appropriate time of application of sweet orange and cassava peels combinedon shoot length of tomato at the plant house.

Analysis of variance

Variate: Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	7185.71	798.41	8.23	<.001
Residual	50	4850.77	97.02		
Total	59	12036.48			

LSD: 11.42

Appendix 38 Effect of appropriate time of application of sweet orange and cassava peels combinedon fresh shoot weight of tomato at the plant house.

Analysis of variance

Variate: Shoot weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	3585.27	398.36	37.53	<.001
Residual	50	530.75	10.61		
Total	59	4116.01			

LSD: 3.8

Appendix 39: Effect of appropriate time of application of sweet orange and cassava peels combinedon fresh root weight of tomato at the plant house.

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Analysis of variance

Variate: Root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	58.0057	6.4451	9.10	<.001
Residual	50	35.4307	0.7086		
Total	59	93.4364			

LSD: 0.976

Appendix 40 Effect of appropriate time of application of sweet orange and cassava peels combinedon number of root-knot nematode juveniles extracted from tomato roots at the plant house.

Analysis of variance

Variate: Number of Juvenile/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	6615.896	735.100	591.46	<.001
Residual	50	62.143	1.243		
Total	59	6678.038			

LSD: 1.293

Appendix 41: Effect of appropriate time of application of sweet orange and cassava peels combinedon number of root-knot nematode juveniles extracted from soil at the plant house.

Analysis of variance

Variate: Number of Nematodes/100ml soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

:

TRT	9	11363.123	1262.569	721.24	<.001
Residual	50	87.528	1.751		
Total	59	11450.650			

LSD: 1.534

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Appendix 42 Number of root-knot nematode as affected by appropriate time of application of sweet orange and cassava peels combinedat the plant house.

Analysis of variance

Variate: Number of eggs/5g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	24088.246	2676.472	834.92	<.001
Residual	50	160.284	3.206		
Total	59	24248.529			

LSD: 2.08

Appendix 43: Reproduction factor of root-knot nematode as affected by appropriate time of application of sweet orange and cassava peels combinedat the plant house.

Analysis of variance

Variate: Reproduction factor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	24.327141	2.703016	1703.70	<.001
Residual	50	0.079328	0.001587		
Total	59	24.406469			

LSD: 0.05

:

Appendix 44 Effect of appropriate time of application of sweet orange and cassava peels combined on root-knot nematode gallingon tomato roots at the plant house.

Analysis of variance

Variate: Gall ndex

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TRT	9	283.0833	31.4537	89.87	<.001
Residual	50	17.5000	0.3500		
Total	59	300.5833			

LSD: 0.69

Appendix 45: Effect of appropriate rate of application of sweet orange and cassava peels combined on fresh root weight of tomato on the field.

Analysis of variance

Variate: Fresh root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.572	1.786	1.57	
REP.*Units* stratum					
TRT	6	362.560	60.427	53.18	<.001
Residual	12	13.634	1.136		
Total	20	379.767			

LSD: 1.9

Appendix 46 : Effect of appropriate rate of application of sweet orange and cassava peels combined on number of fruits of tomato on the field.

Analysis of variance

Variate: Number of fruits/plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.429	2.714	1.00	
REP.*Units* stratum					
TRT	6	284.571	47.429	17.47	<.001
Residual	12	32.571	2.714		
Total	20	322.571			

LSD: 2.9

Appendix 47: Effect of appropriate rate of application of sweet orange and cassava peels combined on fruit size of tomato on the field.

Analysis of variance

Variate: Fruit size at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.04952	0.02476	0.57	
REP.*Units* stratum					
TRT	6	24.02286	4.00381	92.91	<.001
Residual	12	0.51714	0.04310		
Total	20	24.58952			

: Effect of appropriate rate of application of sweet orange and

LSD: 0.67

Appendix 48 cassava peels combined on plant height of tomato on the field.

Analysis of variance

Variate: Plant height at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	20.89	10.45	0.94	
REP.*Units* stratum					
TRT	6	284.39	47.40	4.27	0.016
Residual	12	133.09	11.09		
Total	20	438.37			

LSD: 5.9

Appendix 49: Effect of appropriate rate of application of sweet orange and cassava peels combined on fresh shoot weight of tomato on the field.

Analysis of variance

Variate: Fresh shoot weight at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	130.7	65.4	0.62	
REP.*Units* stratum					
TRT	6	119606.1	19934.4	188.40	<.001
Residual	12	1269.7	105.8		
Total	20	121006.5			

LSD: 18.3

: Effect of appropriate rate of application of sweet orange and

Appendix 50

cassava peels combined on fruit yield of tomato on the field.

Analysis of variance

Variate: Fruit yield per hectare

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	707823.	353911.	0.75	
REP.*Units* stratum					
TRT	6	348896953.	58149492.	122.52	<.001
Residual	12	5695271.	474606.		
Total	20	355300046.			

LSD: 634.0

Appendix 51: Effect of appropriate rate of application of sweet orange and cassava peels combined on number of root-knot nematodes extracted from the soil on the field.

Analysis of variance

Variate: Number of root-knot nematodes/100ml soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	17857.	8929.	2.36	
REP.*Units* stratum					
TRT	6	1764524.	294087.	77.60	<.001
Residual	12	45476.	3790.		
Total	20	1827857.			

LSD: 109.5

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Appendix 52 Effect of appropriate rate of application of sweet orange and cassava peels combined on number of root-knot nematode juveniles extracted from the root of tomato on the field.

Analysis of variance

Variate: Number of juveniles/10g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2615.7	1307.9	1.92	
REP.*Units* stratum					
TRT	6	134668.3	22444.7	32.89	<.001
Residual	12	8188.0	682.3		
Total	20	145472.0			

LSD: 46.5

Appendix 53: Effect of appropriate rate of application of sweet orange and cassava peels combined on number of root-knot nematode eggs extracted from the roots of tomato on the field.

Analysis of variance

Variate: Number of eggs of root-knot nematodes/10g root

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1125.	563.	0.25	
REP.*Units* stratum					
TRT	6	1300966.	216828.	96.50	<.001
Residual	12	26964.	2247.		
Total	20	1329055.			

LSD: 84.3

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Appendix 54 Effect of appropriate rate of application of sweet orange and cassava peels combined on galling of root-knot nematodes on tomato on the field.

Analysis of variance

Variate: Gall index at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0952	0.0476	0.12	
REP.*Units* stratum					
TRT	6	64.2857	10.7143	28.12	<.001
Residual	12	4.5714	0.3810		
Total	20	68.9524			

LSD: 1.1

Appendix 55: Assessment scale for determination of root-knot nematode galling on vegetable crops.

Gall index was assessed using a scale 0-10 (Bridge and Page, 1980)

Were:

0 = No knot on roots; 1 = Few small knots, difficult to find; 2 = small knots only but clearly visible, main roots clean; 3 = some larger knots visible, main roots clean; 4 = larger knots predominate but main roots clean; 5 =50% of roots infested, knotting on some main roots, reduced root system; 6 = knotting on main roots; 7 = majority of main roots knotted; 8 = all main roots, including tap roots visible; 9 = all roots severely knotted, plant usually dying and 10 = all roots severely knotted, no root system, plant usually dead.