

Original Research Article

Efficacy of sweet orange and cassava peel amendments for the management of root-knot nematodes on tomato

Abstract

Aim: To determine efficacy of organic amendments in the management of root-knot nematodes.

Design of the study: Experiments were arranged in a Complete Randomized Design (CRD) with five treatments replicated five times and Randomized Complete Block Design (RCBD) with 11 treatments replicated four times at the laboratory and field respectively.

Study place and duration: The studies were carried out in the Nematology laboratory and research field at the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana from March, 2014 to January, 2015.

Methodology: Five aqueous extracts: 1. Fresh sweet orange peel (FOP), 2. Fresh cassava peel (FCP), 3. Dry sweet orange peel (DOP), 4. Dry cassava peel (DCP) and Sterilized water (control) on egg hatching inhibition and juvenile mortality of root-knot nematodes were studied at the laboratory. Eleven treatments (1) FOP, (2) DOP, (3) FCP, (4) DCP, (5) FCP +FOP, (6) FOP+FCP (7) DOP+DCP, (8) DCP+DOP, (9) NPK and $((\text{NH}_4)_2 \text{SO}_4)$ fertilizers, (10) Carbofuran, (11) No application (control) against root-knot nematodes were studied on the field.

Results: At the laboratory, the highest percentage egg hatching inhibition (87.8%) and juvenile mortality (94.8%) of root-knot nematodes were recorded by using the fresh sweet orange peel FOP aqueous extract. Significant differences ($P < 0.05$) were observed between fresh sweet orange peel FOP aqueous extract and the rest of the treatments. For the field experiment, all the plots were amended with sweet orange and cassava peels out-yielded the application of NPK and $((\text{NH}_4)_2 \text{SO}_4)$ fertilizers. The combined application of fresh sweet orange FOP and cassava peels DOP significantly increased ($P < 0.05$) yield more than the rest of the treatments. Carbofuran, followed by the combined application of fresh sweet orange and cassava peels significantly reduced ($P < 0.05$) gall index, number of eggs, number of root-knot nematode

juveniles in the root and number of nematodes in the soil in both seasons. However, no significant difference ($P>0.05$) was observed among them^[BD4].

Conclusion: The sweet orange and cassava peels were found to be effective in reducing galling and population of root-knot nematodes in the roots and soil of tomato. Also, the aqueous extract of fresh and dry sweet orange and cassava peels showed high potential in egg hatching inhibition and juvenile mortality of root-knot nematodes. Further evaluation of the combined application of fresh sweet orange and cassava peels at farmers' fields is recommended. Also, methods of application, for example, in the form of aqueous extract should be tested.

Keywords: Organic amendments; aqueous extracts; fungal antagonists; inhibition; mortality.

1. INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are among the most polyphagous and damaging genera of plant-parasitic nematodes (1). In the tropics, significant yield loss has been recorded on-in tomato due to root-knot nematode damage and, in some cases, the plants die before reaching maturity (2). The high rate of development and fecundity of these nematode species, make their control difficult to control on crops (3).

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 health and environmental health hazards and therefore, not sustainable. In addition, the economic cost of research and registration of new chemicals are big obstacles 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 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 effective nematode management.

Alternative control techniques, such as organic amendment have been used with some success. The use of organic amendments for management of plant-parasitic nematodes has been

demonstrated in a number of studies (4, 5, 6). Neem seed cake, castor seed cake, castor bean extract, sweet orange and cassava peels [BD5] have been widely studied for their nematicidal properties (4, 7, 5).

Sweet orange [BD6] and cassava are largely consumed in Ghana but the peels end up as wastes [BD7], the disposal of which causes both economic and environmental problems. The effective and sustainable use of sweet orange and cassava peels as organic amendment for root-knot nematode management is highly desirable. Loumédjnon *et al.* (8) reported significant reduction of root-knot nematode in roots and soil of carrot using dry sweet orange and cassava peels in the Republic of Benin. However, limited research has been made on fresh sweet orange and cassava peels and their combination as organic amendments to control root-knot nematodes.

In Ghana, there is limited information on the use of plant materials in the management of plant-parasitic nematodes (9). Farmers mostly depend on cultural practices such as crop rotation, which sometimes ~~do not give~~ fail to yield good result. However, organic amendments have potential of mitigating the effects of plant parasitic nematodes on crops through their chemical composition and/or influence on nematophagous fungi and free-living nematodes. Therefore, the purpose of this study was to evaluate the efficacy of fresh and dry peels of sweet orange and cassava in the management of root-knot nematodes. Also, to evaluate the effect of sweet orange and cassava peels on the ~~nematophagus~~ biocontrol fungi (*Trichoderma* spp.) and free-living nematodes.

2. MATERIALS AND METHODS

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

2.1. Laboratory experiment: Evaluation of sweet orange and cassava peel aqueous extracts for their nematicidal potentials on root-knot nematodes *in vitro*

Complete Randomized Design (CRD) with five replications was used.

The treatments used were aqueous extracts of:

- 1) Fresh sweet orange peel
- 2) Dry sweet orange peel
- 3) Fresh cassava peel
- 4) Dry cassava peel
- 5) Sterilized water (control)

2.1.1 Sources of the organic amendments

Each of the organic amendments was collected from the same source to maintain homogeneity of the materials. Fully ripe fruits of the sweet orange (*Citrus sinensis* [L.] Obsbeck) variety, Valencia were obtained from 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 at Kumasi.

2.1.2 Preparation of the aqueous extracts of the organic amendments for the *in vitro* studies

The sweet orange and cassava peels were cut into small pieces of approximately 0.25cm² 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 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. The equivalent to 50g dry matter of each peel was soaked in 150ml of distilled water (1:3 w/v dilution) for 24h in a beaker at room temperature. The aqueous extract of each type was collected by sieving through cheesecloth. Twenty millilitres (20ml) of each aqueous extract was dispensed into separate 9 cm diameter Petri dish.

2.1.3 Sterilization of the 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 with water and heated on fire at 80°C for 3h. After sterilization, the soil was allowed to cool for 24h before used for the filling of the pots.

2.1.4 Culturing 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 were each filled with 8kg of the steam-sterilized soil mixture and 21-day-old tomato seedlings nursed in sterilized soil were transplanted. The plants were inoculated with root-knot nematode eggs, two weeks after transplanting and allowed to grow for two months at the plant house [BD9]. Two months after inoculation, the plants were uprooted and roots were washed gently under tap water to remove soil and debris. The roots were cut into pieces of about 0.5cm length with a pair of scissors. The root-knot nematodes juveniles were extracted using modified Baermann tray method (10). Extraction of root-knot nematode eggs was done using modified Hussey and Barker method (11).

2.1.5 Assessment of root-knot nematode juvenile mortality in the different aqueous extracts *in vitro*

A total of 100 root-knot nematode juveniles ~~was~~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 24, 48 and 72h after inoculation. The immobilized nematodes ~~that~~-were assumed dead~~th-and~~ were then transferred into Petri dishes containing sterilized water. The Petri dishes were left on the bench under laboratory conditions for another 24h. Nematodes were probed with a sharp-edge needle under a microscope and the ones that were straight in shape and remained immobile even after probing were considered dead (12).

2.1.6 Assessment of root-knot nematode egg hatch inhibition in the different aqueous extracts *in vitro*

Also 100 eggs were counted with the aid of a stereo microscope and placed in each Petri dish containing 20ml of each aqueous extract. The Petri dishes were placed on a laboratory bench at

average temperature of 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 as described above. Square lines were made at the underside of the Petri dish and all the juveniles and eggs in each square were counted.

2.2 Field experiment: Effect of sweet orange and cassava peels and their combinations on root-knot nematodes of tomato in the field

2.2.1 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), located on longitude 01° 33'W and latitude 06° 41'N. The soil was loamy sand Dystric Cambisol with a composition of 87.3% sand, 7.0% silt and 5.7% clay in the topsoil (0-15cm). The pH, percentage organic carbon and total nitrogen were 7.4, 1.2 and 0.06 respectively.

The site was previously grown to tomato, pepper and eggplant. Before planting of the experiment, weed samples were collected and identified. The most predominant weeds were *Cyperus rotundus* (L.) [BD10] and *Panicum maximum* (Jacq.).

2.2.2 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 two seasons at the same location; the first experiment was carried out in the major during the peak rainy season (June to September, 2014) and the second in during the minor off peak rainy season (November, 2014 to January, 2015). The experiment was carried out under natural infestation conditions without any nematode inoculation.

The experiment consisted of the following treatments:

- 1) 50g/plant of fresh sweet orange peel
- 2) 50g/plant of dry sweet orange peel

- 3) 50g/plant of fresh cassava peel
- 4) 50g/plant of dry cassava peel
- 5) Zero application (control)
- 6) 25g/plant of fresh cassava peel + 25g/plant of fresh sweet orange peel
- 7) 25g/plant of fresh sweet orange peel + 25g/plant of fresh cassava peel
- 8) 25g/plant of dry sweet orange peel + 25g/plant of dry cassava peel
- 9) 25g/plant of dry cassava peel + 25g/plant of dry sweet orange peel
- 10) NPK (15:15:15) + Ammonium sulphate ((NH₄)₂ SO₄) fertilizers
- 11) Carbofuran (Chemical nematicide)

2.2.3 Soil preparation for transplanting of tomato seedlings

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

2.2.4 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 dry peels were obtained by drying fresh peels under shade for two weeks and pounded into slightly powdery form using mortar and pestle. The fresh peels were cut into small pieces (approximately 0.25cm³). Each type of peel was applied at the rate of 50g dry matter/plant (BD11)(8).

The single dose treatments were applied four weeks before seedlings were transplanted. The peels were placed on ridges in 10cm diameter circle at 40cm apart and thoroughly incorporated into the soil with a hand fork to a depth of 10cm. The first part of the combined treatments (sweet orange and cassava peels) were applied four weeks before and the second part was applied at three weeks after transplanting. (BD12) The amendments were placed around the plant in the form of a ring at 5cm radius from the base of each plant and incorporated to a depth of 10cm and covered with soil to facilitate decomposition and distribution of the constituents into the soil. Carbodan 3% G carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was applied at four weeks before transplanting at the rate of 0.5g per plant and incorporated as the organic amendment described above. The product was manufactured by Shenzhen Baocheng Chemical Industry, China.

2.2.5 Transplanting of seedlings^[BD13] and application of fertilizers

Four-week-old tomato seedlings (var. Power) were transplanted^[BD14] onto the ridges at a spacing of 50cm intra-row and 90cm inter-row. Vigorous and equal height seedlings were used to maintain uniformity.

NPK (15: 15:15) fertilizer (Zouping Runzi Chemical Industry Co., Ltd., China) was applied at a rate of 250 kg/ha at four weeks after transplanting. In addition, ammonium sulphate [(NH₄)₂ SO₄] was applied at a rate of 125kg/ha (12) at six weeks after transplanting. Both fertilizers (NPK and (NH₄)₂ SO₄) were applied in rings at 5cm radius and 10cm deep from the base of each plant and covered with soil.

2.2.6 Application of fungicide and insecticide

Merpan^[BD15]® 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 30g Bifenthrin/L) was applied every week at the rate of 1L per hectare. Golan® (200g of Acetamiprid/L, Makhteshim Chemical Works Ltd, Israel) was also applied at the rate of 0.5L/ha every two weeks to control whiteflies. All pesticides were thoroughly mixed with water in the knapsack sprayer and applied.

2.2.7 Cultural Practices

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 having contact with soil. Supplementary irrigation^[BD16] was carried out when there was no rainfall during the off season^{in the minor season}.

2.2.8 Extraction of root-knot nematode eggs and juveniles from roots and soil

The tomato plant roots were uprooted at three months after transplanting 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. The roots were cut into pieces with a pair of scissors. Ten grams (10g) of the cut roots from each sample was used to extract root-knot nematode eggs and juveniles. 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 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 (10). The modified method of Hussey and Barker, (11) was used to extract the eggs from the roots.

2.2.9 Morphological identification of nematodes

Nematodes relaxed and preserved in formalin and glycerol (14) were used for identification. During identification, 1ml of the nematode suspension in a tube was collected with a syringe and then placed into a counting tray under the inverted compound microscope (Leica, Leica Microsystems company Ltd., Germany). The pictures of the nematodes were taken and recorded at magnifications 25x and 40x and were later used for identification. 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.

[BD17]

2.2.10 Data collected

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.

2.2.10.1 Number of fruits and yield of tomato

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 ripe (red colour). The yield data collected at different intervals were summed up to have the final data for each treatment.

2.2.10.2 Number of eggs and nematodes in roots and soil at harvest

Soil and root samples were collected at three months after transplanting for extraction of root-knot nematodes as described above. A total of three plants were systematically uprooted from each plot. [BD18]

2.2.10.3 Determination of gall index of root-knot nematodes on tomato roots

Galling were assessed on the roots of tomato plants after harvest using gall index scale of 0-10 (15) described below.

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.

2.2.10.4 Determination of fungal antagonists in the soil [BD19]

Before 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 under shade in the laboratory for 48h before isolation. Fungi were isolated by serial dilution at 10^{-6} [R20] and number of colonies for each fungus was counted and identified. The Potato Dextrose Agar (PDA) was used to isolate the fungi and identified using a compound microscope (Leica, Leica Microsystems company Ltd., Germany), specimen and reference manuals (16). Part of the same samples ~~was~~ ~~ere~~ used to extract and determine the population of free-living nematodes.

2.2.11 Data analyses

The data collected from the experiment were subjected to ANOVA [BD21] using GenStat. 12th edition software. The means were compared using LSD at $P < 0.05$. Nematode and egg counts were square root transformed [BD22] $\sqrt{(x + 1)}$ where x is the mean count before statistical analysis and back-transformed.

3. RESULTAND DISCUSSION

3.1 Laboratory experiment: Evaluation of sweet orange and cassava peels for their nematocidal potentials *in vitro*

3.1.1 Percentage eggs hatched at 3, 6 and 9 days after inoculation in different aqueous extracts *in vitro*

The effect of organic amendments (sweet orange and cassava peels) on root-knot nematode egg hatch inhibition at 3, 6 and 9 days after application is presented in Table 1. There were significant differences ($P<0.05$) between aqueous extract of fresh sweet orange peel and the other extracts in all the observation dates except fresh cassava peel aqueous extract at three days after inoculation (Table 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 1).

Table 1. Percentage of eggs hatch inhibition at 3, 6 and 9 days after inoculation in different aqueous extracts^[BD23]

Treatment	Percentage egg hatch inhibition/ days after inoculation		
	3	6	9
Dry cassava peel	85.1	72.6	70.1
Dry sweet orange peel	89.6	76.6	74.8
Fresh cassava peel	91.0	81.0	79.1
Fresh sweet orange peel	93.1	88.4	87.8
Sterilized water (control)	54.2	10.5	1.9
LSD (0.05)	2.2	5.7	5.1

CV (%)

2.1

8.3

8.0

The highest percentage of egg hatch inhibition in the fresh sweet orange peel aqueous extract may be attributed to the presence of phytochemicals such as limonene which suppressed egg hatching. According to Isman *et al.* (17) limonene 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. Osei *et al.* (18) reported that citrus peels inhibited egg hatching by more than 90% after 72h of exposure *in vitro*. Also, the fresh cassava peel aqueous extract significantly inhibited egg hatching than dry cassava peel aqueous extract and sterilized water. This could be due to the presence of hydrogen cyanide which is known to have nematicidal properties (19). All the peels studied were found to significantly inhibited the hatching of nematode eggs hatching more than the when compared to control. The effect of the organic amendments on egg hatching can be attributed to the permeability of the eggs due to the pore-spaces through which phytochemicals pass to suppress hatching. According to Perry *et al.* (19), evolution in the eggshell structure of *Meloidogyne* spp. before hatching are-is manifested by a marked change in permeability. When eggshells are permeable, the unhatched second stage juveniles are susceptible to toxic compounds, including plant extracts that may have potential as control agents (19).

3.1.2 Percentage mortality of root-knot nematode juveniles in the different aqueous extracts *in vitro*

Table 2 shows the effect of aqueous extracts of the organic amendments on the mortality of root-knot nematode juveniles at 12, 48 and 72h after application. The result of the percentage mortality followed a similar trend in all the observation periods. The aqueous extract of fresh sweet orange peel had significantly higher ($P < 0.05$) nematode mortality in all the three periods observed (45.6, 86.6 and 94.8%), followed by fresh cassava peel aqueous extract (36.2, 71.0 and 86.6%) and dry sweet orange peel aqueous extract (35.4, 70.6 and 86.4%) at 12, 48 and 72h respectively (Table 2).^[BD24]

Table 2. Percentage mortality of root-knot nematode juveniles in different aqueous extracts *in vitro*

Treatment	Percent mortality/ hours after inoculation		
	12	48	72
Dry Cassava Peel	22.0	57.0	62.6
Dry Sweet orange Peel	35.4	70.6	86.4
Fresh Cassava Peel	36.2	71.0	86.6
Fresh Sweet orange Peel	45.6	86.6	94.8
Sterilized water (control)	0.0	0.0	0.0
LSD (0.05)	1.2	1.1	2.0
CV (%)	3.1	1.2	1.6

There were significant differences ($P < 0.05$) among treatments under each of the observation periods (12, 48 and 72h) (Table 2). Significant differences ($P < 0.05$) were observed between aqueous extract of fresh sweet orange peel and the rest of the treatments applied (Table 2). Tsai (20) reported that aqueous extract of sweet orange peels caused mortality rate of root-knot nematode juveniles by 93.5% after 72h of exposure. The highest mortality rate in the fresh sweet orange peel aqueous extract could be due to higher stability of lemonene in water compared to hydrogen cyanide. Li and Chang (21) stated that lemonene moderately dissolved in water and remained stable for eight weeks after storage, while hydrogen cyanide had low persistence in water. Also Li *et al.* (22) reported that lemonene, which is one of the major constituents in sweet orange peel essential oil exhibited strong nematicidal activity against *M. incognita*.

Comparing the fresh and dry peels, it was observed that fresh peels of sweet orange and cassava had higher effects on egg hatching and mortality of root-knot nematodes compared to the dry peels. The higher inhibition effect on egg hatching and mortality of juveniles by the fresh peels aqueous extracts may be due to higher concentration of phytochemicals (lemonene and hydrogen cyanide) compared to the dry ones. Kamal *et al.* (23) have shown that concentration of lemonene is higher in the fresh peels compared to the dry peels. The concentration of limonene was found

to be significantly higher in the fresh peel of sweet orange (80.9 %) compared to the dry peels (66.8 %) (23). [BD26]

3.2 Field experiment : Effect of sweet orange and cassava peels and their combinations on root-knot nematodes of tomato in the field

The initial population of 686 juveniles [BD27] of root-knot nematodes/100ml soil was observed before the application of the various treatments in the field.

3.2.1 Effect of sweet orange and cassava peels on gall index, number of eggs, number of juveniles of root-knot nematodes extracted from the roots and soil of tomato in the major and minor seasons

The effects 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 Tables 3 and 4. 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 fresh sweet orange and cassava peels. However, the gall index and number of eggs of root-knot nematodes were significantly lower ($P<0.05$) in the application of carbofuran and combined application of fresh sweet orange and cassava peels compared with the other treatments (Tables 3 and 4). Similarly, no significant differences ($P>0.05$) were observed between the application of fresh sweet orange peel alone and combined application of dry sweet orange and cassava peels (Tables 3 and 4). The significant reduction of gall index and number of eggs in the combined application of fresh sweet orange and cassava peels were found to be as effective as carbofuran. This might be attributed to the nematicidal properties in the sweet orange and cassava peels. Lemonene in sweet orange has been found to be nematicidal and nematostatic on the hatching and mortality of *M. graminicola* (24). Higher mortality and reduction in galling of root-knot nematodes were observed after incorporation of cassava peels into the soil planted with pepper (25). In addition, low galling and number of eggs on the roots

could be associated with the potential of the combined application of fresh sweet orange and cassava peels in reducing the population (Tables 3 and 4). Hussain *et al.* (26) observed a positive correlation between the number of galls and egg masses and population density of root-knot nematodes in the roots of tomato.

Table 3. Gall index, number of eggs, number of juveniles extracted from the root and soil of tomato in the major season

Treatments	^x Gall index	^z Number of eggs/10g root	^z Number of juveniles/10g root	^z Number of juveniles/100ml soil
Fresh Sweet orange Peel (FOP)	2.5	1061	117	379
Dry Sweet orange Peel (DOP)	2.5	1183	131	408
Fresh Cassava Peel (FCP)	2.8	1278	128	432
Dry Cassava Peel (DCP)	3.8	1388	129	475
FCP + FOP	1.8	873	75	329
FOP + FCP	1.3	816	65	280
DOP +DCP	2.5	1124	130	383
DCP + DOP	2.5	1169	132	395
NPK (15:15:15)+((NH ₄) ₂ SO ₄) fertilizers	5.0	1507	185	970
Carbofuran	1.3	797	63	260
Non application (control)	6.3	1521	205	1078
LSD (0.05)	0.4	81.2	16.0	81.1
CV(%)	8.1	2.4	3.9	5.5

^z All count data were back-transformed after transformation using $\sqrt{(x+1)}$;

^x Gallings on scale 0-10, where 0 = no galls and 10 = root system completely galled

Table 4. Gall index, number of egg and juveniles of root knot nematodes extracted from the root and soil of tomato at the field in the minor season

Treatments	^x Gall index	^z Number of eggs/10g root	^z Number of juveniles/10g root	^z Number of juveniles/100ml soil
Fresh Sweet orangePeel (FOP)	1.9	831	108	278
Dry Sweet orangePeel (DOP)	2.3	949	133	343
Fresh Cassava Peel (FCP)	2.8	969	136	365
Dry Cassava Peel (DCP)	3.0	1178	143	513
FCP + FOP	1.0	709	99	235
FOP + FCP	1.0	670	88	190
DOP +DCP	2.3	852	127	313
DCP + DOP	2.3	880	132	324
NPK (15:15:15)+ ((NH ₄) ₂ SO ₄) fertilizers	7.0	1725	218	1018
Carbofuran	1.0	635	85	175
No application (control)	7.3	1737	226	1113
LSD (0.05)	0.5	48.6	10.8	44.9
CV (%)	5.6	3.2	3.0	3.2

^z All count data were back-transformed after transformation $\sqrt{(x+1)}$;

^x Gallings on scale 0-10, where 0 = no galls and 10 = root system completely galled

The number of root-knot nematode juveniles in the roots and soil were also significantly reduced ($P<0.05$) in the carbofuran and fresh sweet orange and cassava peels combination treated plots compared with the other treatments. However, carbofuran was not significantly different ($P>0.05$) from the combined application of fresh sweet orange and cassava peels (Tables 3 and 4). Also, the number of root-knot nematode juveniles in the roots and soil were significantly

reduced in all the other treatments ($P < 0.05$) compared with the application of NPK (15:15:15) and $((\text{NH}_4)_2 \text{SO}_4)$ fertilizers and control (Tables 3 and 4). Generally, combined application of fresh sweet orange and cassava peels performed better than the sole application of the peels in all the parameters measured.

The combined application of fresh sweet orange and cassava peels was found to be as good as carbofuran in controlling root-knot nematode. [BD28] Incorporation of citrus peels in the soil highly suppressed plant parasitic nematodes in pineapple production (27). Similarly, Loumédjinon *et al* (8) indicated that dry sweet orange and cassava peels significantly reduced the number of nematodes in soil and roots and percentage roots galled in carrot compared to control. Also, John *et al*, (28) stated that *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. Therefore, the combination of fresh sweet orange and cassava peels could have caused the high reduction of root-knot nematode juveniles in the roots and soil of tomato. This result agrees with that of Sundararaju and Kumar (29) who reported that the integration of organic amendment and green manure was more effective in reducing the number of nematodes and subsequently, increased plant growth and yield than the organic treatment alone.

3.2.2 Effect of the treatments on *Trichoderma* sp. and free-living nematodes in the soil before and after application of treatments

The initial population of nematophagus fungus, bacterivorous and fungivorous nematodes are presented in Table 5.

Table 5 Fungus and free-living nematodes isolated from the experiment before treatment application

No. of fungus and free-living nematodes			
Nematophagus fungus	Bacterivorous		Fungivorous
<i>Trichoderma</i> [R29] sp.	<i>Heterocephalobellus</i> sp.	<i>Eucephalobus</i> sp.	<i>Ditylenchus</i> sp.
2[BD30]	9	8	11

The types of microorganisms predominantly found in the soil were *Trichoderma* sp. as nematophagus [BD31] fungus, *Heterocephalobellus* sp. and *Eucephalobus* sp. as bacterivorous nematodes and *Dictylenchus* sp. as fungivorous nematode after application of treatments (Table 6). The combined application of fresh sweet orange and cassava peels significantly increased ($P < 0.05$) number of colonies of *Trichoderma* sp. (9 colonies), followed by the application of fresh sweet orange peel alone (8 colonies) (Table 6). This might be due to the presence of certain chemical compounds that favours growth [BD32] and multiplication of *Trichoderma* sp. compared to the other treatments (cassava peels, NPK (15:15:15) and $((\text{NH}_4)_2 \text{SO}_4)$ fertilizers and carbofuran). According to Duli *et al.* (30) results obtained from laboratory experiment on the evaluation of different substrates for *T. harzianum* showed highest growth rate and sporulation on sweet orange peel substrate compared to other substrates tested. Also, previous research has shown that *Trichoderma* sp., have good potentials for in controlling plant parasitic nematodes control. Khan *et al.* (31) 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 the eggplant. This must have contributed to the low population and damage of root-knot nematode observed in the sweet orange peels amended plots (Tables 3 and 4).

Table 6. Fungus and free-living nematodes isolated from different soil treatments and their trophism

Treatment	No. of fungus and free-living nematodes [BD33]			
	Nematophagus fungus	Bacterivorous		Fungivorous
	<i>Trichoderma</i> sp.	<i>Heterocephalobellus</i> sp.	<i>Eucephalobus</i> sp.	<i>Dictylenchus</i> sp.
	After application	After application	After application	After application
Fresh Sweet orange Peel (FOP)	8	1	1	2
Dry Sweet orange Peel (DOP)	6	0	1	1
Fresh Cassava Peel (FCP)	2	24	18	23
Dry Cassava Peel (DCP)	1	17	16	25
FCP +FOP	8	8	7	5

FOP + FCP	9	2	2	3
DOP + DCP	7	1	1	2
DCP + DOP	6	5	4	6
NPK (15:15:15) and $((\text{NH}_4)_2 \text{SO}_4)$ fertilizers	2	4	3	2
Carbofuran	1	0	1	0
No application (control)	1	3	4	3
LSD (0.05)	1.2	0.9	1.3	0.8
Cv (%)	5.3	6.7	5.1	4.8

In the case of bacterivorous and fungivorous nematodes, the highest counts were observed in application of fresh and dry cassava peels compared with fresh and dry sweet orange peels and their combinations (Table 6). The populations of bacterivorous and fungivorous nematodes extracted from the soil after application of in carbofuran and fresh and dry sweet orange peels treated soils were the treatments were found to be significantly reduced lower ($P < 0.05$) compared to untreated soils in the application of carbofuran and plots treated with fresh and dry sweet orange peels (Table 6). Odeyemi *et al.* (32) reported that addition of organic amendments in the soil had resulted in population increase of free-living nematodes. Potential changes in the abundance and community structure of free-living nematodes were observed following the application of organic amendments in the surveyed areas (33). Although, the cassava peel contains cyanide which kills nematode (8) however, cyanide is easily degraded under high temperature and in water (22). This low persistence of cyanide in cassava peels might have favoured the rapid multiplication of the bacterivorous and fungivorous nematodes at where fresh and dry cassava peel was applied.

3.2.3 The effects of sweet orange and cassava peels on the yield of tomato over two seasons

The yields of tomato were significantly higher ($P < 0.05$) in the combined application of fresh sweet orange and cassava peels than the rest of the treatments over the two seasons (Table 7).

Table 7 Effect of sweet orange and cassava peels on fruit yield of tomato over two seasons.

Treatments	Yield (kg/ha)	
	Major seasonPeak rainy season	Minor seasonOff season
Fresh Sweet orange Peel (FOP)	19212.1	15288.5
Dry Sweet orange Peel (DOP)	18171.9	14373.1
Fresh Cassava Peel (FCP)	17507.9	14660.1
Dry Cassava Peel (DCP)	17475.8	12394.0
FCP +FOP	20774.3	16170.0
FOP + FCP	20981.1	16707.1
DOP +DCP	18832.5	14057.3
DCP + DOP	18547.3	14008.1
NPK (15:15:15) +((NH ₄) ₂ SO ₄) fertilizers	15926.2	9943.2
Carbofuran	19066.2	15154.7
No application (control)	6511.6	3311.1
LSD (0.05)	834.6	662.2
CV (%)	3.2	3.5_[F34]

522

523 The highest yield observed in the combined application of fresh sweet orange and cassava peels

524 can be explained by the low galling and root-knot nematode population (Tables 3 and 4). The

525 level of reduction in quantity and/or quality of yield of crops due to root-knot nematode damage

526 is positively correlated to their population (31). Ononuju *et al.* (34) reported that dry cassava

527 peels performed as good as carbofuran in reducing gall index and population of root-knot

528 nematodes in the roots and soil of okra. Also essential oil and aqueous extracts of citrus peels

529 were found to inhibit hatching, migration and induce mortality of *Meloidogyne incognita* (35).

530 The combination of fresh sweet orange and cassava peels could have reduced the damage of

531 root-knot nematodes on tomato. Ram *et al.* (36) reported that combined application of mustard

and castor cakes as soil amendment significantly reduced population of root-knot nematode (*Meloidogyne incognita*) and improved plant growth parameters of okra more than single treatments. Furthermore, the high presence of *Trichoderma* sp., in the sweet orange peel plots might have helped to significantly reduce the root-knot nematode population density and damage and correspondingly increased yield of tomato in the field. The application of *Trichoderma harzianum* in neem cake amended soil was highly effective against *M. incognita* and resulted into better plant status of tomato Kumar and Khanna (37). The effect of combined application of fresh sweet orange and cassava peels on the yield of tomato was observed to have higher consistent trend over the major and minor seasons.

4. Conclusion

Application of sweet orange and cassava peels were effective in the management of root-knot nematodes in tomato. Percentage egg hatch inhibition and mortality occurred upon application of sweet orange and cassava peels. Also, galling, number of eggs and juveniles in the roots of tomato and soil were effectively reduced when soil was amended with sweet orange and cassava peels. Application of sweet orange peels enhanced the population of *Trichoderma* sp. as nematophagous fungus, while cassava peels enhanced the population of bacterivorous and fungivorous nematodes. The study recommended for multi-location testing of the combined application of fresh sweet orange and cassava peels at various tomato farms. Also, different methods of application such as application in the form aqueous extract should be evaluated to make the technology more appropriate for the farmers.

References

1. Elling, AA. Major Emerging Problems with Minor *Meloidogyne* Species
Phytopathology. 2013; 103:1092-1102
2. Singh, SK, and Khurma RK. Susceptibility of six tomato cultivars to *Meloidogyne incognita*. *South Pacific Journal of Natural Sciences*. 2007; 13: pp.73-77,
3. Crow, WT and Dunn, RA. Introduction to Plant Nematology. 2005;
entnemdept.ufl.edu/crow.htm

4. Oka,Y. Mechanisms of nematode suppression by organic soil amendments – A review. *Applied Soil Ecology*.2010; 44:101–115.
5. Ribeiro, JPN and Lima, MIS. Allelopathic effects of sweet orange (*Citrus sinensis* L.) peel essential oil. *Acta Botanica Brasilica*. 2012; 26(1): 256-259.
6. Osei, K, Addico R, Nafeo A, Edu-kwarteng, A, Agyemang A, Danso Y and Sackey-Asante J. Effect of some organic waste extracts on hatching of *Meloidogyne incognita* eggs. *African Journal of Agricultural Research*. 2011; 6(10): 2255-2259.
7. Adomako,J and Kwoseh, CK. Effect of castor beans (*Ricinus communis* L.) aqueous extracts on the performance of root-knot nematodes on tomato (*Solanum lycopersicum* L.). *Journal of Science and Technology*. 2013; 33(1): 1-11.
8. Loumédjinson,S, Godonou I, Atcha-Ahowé C, James B, Baimey H, Coyne D, and Ahanchédé A. Management of root-knot nematodes on *Solanum macrocarpon* using botanicals in Benin *International Society for Horticultural Science. Acta Horticulturae* 2006 752
9. Osei, MK, Akormah R, Shilh SL, and Green SK. Evaluation of some tomato Germplasm for Resistance to Tomato Yellow Leaf Curl Virus disease (TYCV) in Ghana. *Aspects Applied. Biology*. 2010 96:315-323.
10. Whitehead, AG and Hemming, JR. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Applied Biology* 1965 55:25–38
11. Hussey, RS and Barker, KR. A comparison of methods of collecting inocula of *Meloidogyne* spp.Including a new technology.*Plant Disease Report*. 1973. 57: 1025-1028.
12. Amin, N. Untersuchungen über die Bedeutung endophytischer Pilze für die biologische Bekämpfung des wandernden Endoparasiten *Radopholus similis* (Cobb) Thorne an Bananen, *PhD-Thesis, Bonn University* [BD36]1994
13. Yeboah, S, Berchie, JN, Asumadu, H, Agyeman, K and Acheampong, PP. Influenced of inorganic fertilizer products on the growth and yield of tomatoes (*Lycopersicon esculentum* Mill) *Journal of Experimental Biology and Agricultural Science Volume* 2014; ISSN No. 2320–869
14. Coyne DL, Nicol JM and Claudius-cole B Practical Plant Nematology: A field and laboratory guide. 2007; ISBN 978-131-294-7. Pp58-59
15. Bridge J, and Page SLJ Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest Management*. 1980; 26:296-298.

16. Watanabe,T. Pictorial Atlas of Soil and Seed Fungi. Morphologies of cultured fungi and key to species. 2nd edition. CRC Press LLC. NW Corporate Blvd., Boca Raton, Florida. 2000; pp.42-235.
17. Isman MB, Wilson JA and Bradbury R Insecticidal activities of commercial rosemary oils (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* in relation to their chemical composition. *Pharmaceutical Biology*. 2008; 46:82–87.
18. Osei K, Moss R, Nafeo A, Addico R, Agyemang A, Danso Y and Asante JS. Management of plant parasitic nematodes with antagonistic plants in the forest-savanna transitional zone of Ghana. *Journal of Applied Biosciences*. 2011; 37: 2491-2495.
19. Perry RN, Moens M and Starr JL) Root Knot Nematodes. *CAB International*, 2009. ISBN 13:978 1 84593 492 7
20. Tsai BY . Effect of peels of lemon, orange, and grapefruit against *Meloidogyne incognita*. *Plant Pathology Bulletin*. 2008. 17: 195-201
21. Li PH and Chiang BH Process optimization and stability of cyanide and D-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. 2011 19(1):192-7. doi: 10.1016/j.ultsonch.2011.05.017
22. Li , HQ, Liu, QZ, Liu, ZL , Du, SS and Deng, ZW Chemical Composition and Nematicidal Activity of Essential Oil of *Agastache rugosa* against *Meloidogyne incognita*. ISSN 1420-3049 2013 www.mdpi.com/journal/molecules
23. Kamal G M, Anwar F, Hussain AI., Sarri N and Ashraf M. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal*. 2011; 18 (4): 1275-1282
24. Steffen, CB,Antoniolli, RB, Bosenbecker, ZI, Steffen, VK,. Luparini, GPK, Campos, M. Evaluation of essential oils of medicinal plants for the control of *Meloidogyne graminicola* in flooded rice, *Nematologia Brasileira*. 2008; 22-126-134.
25. Maina, YT, Mohammed, FK and Galadima, IB. The use of organic manure in the management of plant-parasitic nematode in Nigeria. *Journal of Environmental Issues and Agriculture in Developing Countries*. 2012; Vol 4. No 1

26. Hussain, MA, Mukhtar, T, Kanyani, MZ. Assessment damage caused by *Meloidogyne incognita* on okra (*Abelmoschus esculentus*). *Journal of Animal and Plant Science*, 2011; 21(4):857-861.
27. Osei K, Agyemang A, Asante JS, Moss, R and Nafeo A. Nematode suppression and yield improvement potential of organic amendments in pineapple production. International Pineapple Symposium. *Acata Horticulture*. 2011; 902 ISH.
28. Johns S, Chitra, KR, Nambisan B and Mohandas,C, Nematicidal action of cassava cyanogens on root-knot nematode (*Meloidogyne incognita*). *Indian Journal for Nematology*. 2009;11: 57 – 60
29. Duli HF, Khdiar Liqaa, MY and AL-Janaby JA. Use Agro-Waste as a Culture media for Sporulation and conidia Production of *Trichoderma harzianum*. *Plant Disease*. 2013 Volume : 3 Issue : 8 ISSN - 2249-555X
30. Sundararaju P and Kumar V Management of *Pratelenchus coffeae* through organic and inorganic amendments. *InforMusa*. 2012. Vol.12.No 1
31. Khan, MR, Mohiddin, FA, Ejaz1 MN and Khan MM. Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. *Turkey Journal for Biology*. 2010; 36 (2012) 161-169 doi:10.3906/biy-1008-72
32. Odeyemi IS, Afolami SO and Daramola FY Screen house and field investigations of *arbuscular mycorrhiza* and organic fertilizer for the control of the root-knot nematode, *Meloidogyne incognita* infecting cowpea in south western, Nigeria. *Journal of Agricultural Science and Environment*. 2010; ISSN 2277-2755
33. Khodorova, NV and Boitel-Conti M. The Role of Temperature in the Growth and Flowering of Geophytes. *Plants* 2013; 2, 699-711; doi:10.3390/plants2040699
34. Ononuju, CC, Ikwunagu, EA, Okorocho, AD and Okorie, CC. Effect of different agricultural wastes and botanicals on root-knot nematodes (*Meloidogyne* spp) on okra (*Abelmoschus esculentus* L. Moench). *Journal of Entomology and Nematology*. 2014; Vol. 6(5) pp 56-61
35. Ibrahim, SK, Traboulsi, AF and El-Haj S. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*, *Phytopathologia Mediterranea*. 2006; 45 238-246.

- 702
703 36. Ram, L, Siddiqui, AU and Parihar, A. Management of root-knot nematodes,
704 *Meloidogyne incognita* infecting okra using oil cakes. *India Journal of Nematology*.
705 2009; 39:125-127
- 706 37. Kumar, S and Khanna, AS. Role of *Trichoderma harzianum* and neem cake separately
707 and in combination against root-knot nematodes on tomato. *India Journal of*
708 *Nematology*. 2006; 36:247-24
709
710
711
712