

Original Research Article

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

Abstract

Aims: to determine efficacy of organic amendment in the management of root-knot nematode.

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 December, 2015.

Methodology: Five aqueous extracts (1.Fresh orange peel (FOP), 2. Fresh cassava peels (FCP), 3. Dry 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 in the laboratory and Eleven treatments ((1) FOP, (2) DOP, (3) FCP, (4) DCP, (5) FCP +FOP, (6) FOP+FCP (7) DOP+DCP, (8) DCP+DOP, (9) NPK alone, (10) Carbofuran, (11) No application (control) against root-knot nematodes were studied on the field.

Results: At the laboratory, the highest percentage of egg hatching inhibition (87.8%) and juvenile mortality (94.8%) of root-knot nematode were recorded in the fresh orange peel aqueous extract. Significant differences ($P<0.05$) were observed between fresh orange peel aqueous extract and the rest of the treatments. For the field experiment, all the amended plots out-yielded the application of NPK fertilizer. The combined application of fresh orange and cassava peels significantly increased yield more than the rest of the treatments. . The carbofuran, followed by the combined application of fresh orange and cassava peels significantly reduced gall index, number of eggs, number of root-knot nematodes juveniles in the root and number of nematodes in the soil in both seasons. However, no significant difference ($P>0.05$) was observed between them.

Conclusion: At the laboratory, fresh orange peel aqueous extract showed high potential in egg hatching inhibition and juvenile mortality. The combined application of fresh orange and cassava peels performed as good as carbofuran in reducing root-knot nematode population and damage at the field. Further evaluation of the combined application of fresh 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: *efficacy, organic amendment, Meloidogyne spp,*

1.0 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 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 them 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 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 organic amendment have been used with some success. The use of organic amendments for management of plant-parasitic nematodes has been demonstrated in a large number of studies (4). Neem seed cake, castor seed cake, orange and cassava peels have been widely studied for their nematicidal properties (4).

Orange and cassava are largely consumed in Ghana but the peels end up as wastes, which cause both economic and environmental problems. The effective and sustainable alternatives for using orange and cassava peels wastes as organic amendment for root-knot nematode management are highly desirable. Loumédjinon *et al.* (5) 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 made on fresh 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 (6). Farmers mostly depend on cultural practices such as crop rotation for which sometimes do not give good result. 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.

1.1 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.

1.1.1 Experiment 1: Evaluation of sweet orange and cassava peels for their nematocidal potentials *in vitro*

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

The treatments used were aqueous extracts of:

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

1.1.2. Sources of the organic amendments

Each of the organic amendments was collected from the same source to maintain homogeneity of the materials. Fully riped fruits of the sweet orange variety, Valencia (*Citrus sinensis* [L] Obsbeck) were collected from Council for Scientific and Industrial Research-Crop Research Institute (CSIR-CRI), Fumesua, Kumasi, Ghana. The cassava variety “Debo” was obtained from a farmer’s field at Kumasi.

1.1.3 Preparation of the aqueous extracts of the organic amendments

The orange and cassava peels were chopped into small pieces of approximately 0.25cm each. A weight 50g of each peel (sweet orange and cassava) was used. Some of the fresh peels were dried under shade for two weeks to obtain the dry peels. The weights of dry and fresh peels were standardized using the dry matter content. A sub-sample of 100g of fresh and dry peels of sweet orange and cassava were oven-dried at 70° C for 72h and then weighed to determine the dry matter content. The dry matter equivalent of 50g of each peel was soaked in 150ml of distilled water (1:3 w/v dilution) for 24h. The aqueous extract was collected by sieving through cheesecloth. Twenty millilitres (20ml) of each aqueous extract was dispensed into separate 9cm diameter Petri dish.

1.1.4 Sterilization of soil for culturing of root-knot nematodes

Top soil was mixed with river sand at a ratio of 3:1. The soil was sieved with 2ml diameter sieve to remove debris and stones. The soil was placed in a metal barrel and heated on fire at 80°C for 3h. After sterilization, the soil was allowed to cool down for 24h before filling the pots.

1.1.5 Culturing and extraction of root-knot nematode juveniles and eggs

In order to obtain pure *Meloidogyne* spp. (Root-knot nematodes) culture, egg masses were collected from infested-tomato roots from a farmer’s field near KNUST. The plastic buckets were filled with 8kg steam-sterilized soil and 21 day-old tomato seedlings 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. After two months, the plants were uprooted

and roots were washed gently under tap water to remove soil and debris. The roots were cut into pieces of 0.5cm length with a pair of scissors. The root-knot nematodes juveniles were extracted using modified Baermann tray method (7). Extraction of root-knot nematode eggs was done using modified Hussey and Barker, (8) method.

1.1.6. Assessment of root-knot nematode juvenile mortality under different aqueous extracts

A total of 100 root-knot nematode juveniles was placed in each Petri dish containing the 20ml of the aqueous extract from each treatment. The number of dead root-knot nematodes was determined at 24, 48 and 72h after inoculation. The nematodes from each treatment were rinsed with sterilized water after concentrating them using 500µm mesh sieve. The nematodes were then transferred back into the petri dishes containing sterilized water. The petri dishes were left on the bench under laboratory conditions for extra 24h. Nematodes were probed with a sharp-edge needle under the microscope and the ones that were straight in shape and remained immotile even after probing were considered dead (9).

1.1.7. Assessment of root-knot nematode egg hatch inhibition under different aqueous extracts

Also 100 eggs were counted using 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. Square lines were made at the underside of the Petri dish and all the juveniles and eggs in each square were counted. At the end of the counting, the effectiveness of each treatment was determined using the formulae described below:

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158 Percent juvenile mortality = $\frac{\text{Number of dead juveniles}}{\text{Total number of juveniles inoculated (100)}} \times 100$ (10)

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162 Percent inhibition of egg hatching = $\frac{\text{Total number of eggs inoculated} - \text{No of eggs hatched}}{\text{Total number of egg inoculated (100)}} \times 100$

163 (10) **1.2 Experiment 2: Effect of sweet orange and cassava peels and** 164 **their combinations on root-knot nematodes of tomato in the field**

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167 **1.2.1 Material and Methods**

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169 **1.2.2 Description of the experimental location**

170 The experimental site was at the Department of Horticulture, KNUST located on longitude 01°
171 33'W and latitude 06° 41'N. The soil was loamy sand Dystric Cambisol with a composition of
172 87.3% sand, 7.0% silt and 5.7% clay in the topsoil (0-15cm). The pH, percentage organic carbon and
173 total nitrogen were 7.4, 1.2 and 0.06, respectively

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

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178 **1.2.3 Experimental design**

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180 The experiment was arranged in Randomized Complete Block Design (RCBD) with four
181 replications. Plot size was 2 x 4m with walk-way of 1m between plots and blocks. The
182 experiment was repeated for two seasons at the same location; the first experiment was carried
183 out in the major rainy season (June to September, 2014) and the second in the minor rainy season
184 (November, 2014 to January, 2015).

185

186 The experiment consisted of the following treatments:

187 1) 50g/plant of fresh orange peel

188 2) 50g/plant of dry 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 orange peel
- 7) 25g/plant of fresh orange peel + 25g/plant of fresh cassava peel
- 8) 25g/plant of dry orange peel + 25g/plant of dry cassava peel
- 9) 25g/plant of dry cassava peel + 25g/plant of dry orange peel
- 10) NPK fertilizer (15:15:15) alone
- 11) Chemical nematicide (Carbofuran)

1.2.4 Land preparation for transplanting

Weeds at the experimental site were cleared using cutlass and ridges were raised to the height of 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.

1.2.5 Preparation and application of the organic amendments and carbofuran to the soil

Both chopped 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 chopped into small pieces (approximately 0.25cm). Each type of peel was applied at the rate of 50g dry matter/plant (5).

The single 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 combined treatments were applied four weeks before and 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 incorporated to a depth of 10cm and covered with soil to facilitate decomposition and distribution of the constituents into the soil.

The Carbodan 3 % G carbofuran (*2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate*) was applied at 4 weeks before transplanting at the rate of 0.5 gram per plant and incorporated as

the organic amendment described above. The product was manufactured by Shenzhen Baocheng Chemical Industry, China.

1.2.6 Transplanting of seedlings and application of fertilizers

Four weeks-old tomato seedlings (var. Power) were transplanted 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 fertilizer (15: 15:15) (Zouping Runzi Chemical Industry Co., Ltd., China) was applied at a rate of 250kg/ha at four weeks after transplanting. In addition, ammonium sulphate ((NH₄)₂ SO₄) was applied at a rate of 125kg/ha (11) 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.

1.2.7 Application of fungicide and insecticide

Merpan 50 WP (ai Captan 500g/kg) and Folpan 50 WP (ai Folpet 500g/kg) (Makhteshim Chemical Works Ltd, Israel) fungicides were applied at the rate of 1.2kg/ha using knapsack sprayer. The products were thoroughly mixed with water in the prayer before application.

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 used to control whiteflies. The Golan was applied at the rate of 0.5l/ha using knapsack sprayer at every two weeks.

1.2.8 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 was carried out when there was no rainfall.

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

The tomato plant roots were uprooted after harvest and taken to 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 grammes (10g) of the chopped roots from each sample was used to extract root-knot nematode eggs and juveniles as described above. 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 was weighed from each sample using electronic scale (Maestro CKX53, Myweight Co. Ltd, USA). The root-knot nematodes were extracted from the soil using Baermann tray method (7).

1.2.10 Morphological identification of nematodes

Nematodes preserved in formalin and glycerol (12) were used for identification. During identification, 1ml of the nematode suspension in the tube was collected with a syringe and then placed into the counting tray under the inverted compound microscope (Leica, Leica Microsystems company Ltd, Germany). The picture of the nematodes were taken under magnification (25x and 40x) 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.

1.2.11 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.

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

1.2.11.2 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 labelled and placed in plastic container and sent to the laboratory for extraction.

1.2.11.3 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 (13) 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.

1.2.11.4 Determination of fungal antagonists in the soil

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 for two days before isolation. Fungi were isolated by serial dilution at 10^{-6} 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, specimen and reference manuals (14).

1.2.12 Data analyses

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

2.1 Experiment 1. Results and discussion

2.1.1 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 hatching at 3, 6 and 9 days after application is presented in Table 1. The lowest percentage of

egg hatched was observed in the fresh orange peel aqueous extract with 6.9, 11.6 and 12.2% at 3, 6 and 9 days, respectively. The highest percentage of root-knot nematode egg hatched (45.8, 89.5 and 98.2%) was obtained in the sterilized water at the same observation dates.

For the egg hatching inhibition, highest percentage (87.8%) was observed in the fresh orange peel aqueous extract, followed by fresh cassava peel aqueous extract (79.1%). The lowest percentage of egg hatching inhibition (1.8%) was recorded in the sterilized water (Table 1).

Table 1. Percentage of eggs hatched at 3, 6 and 9 days after inoculation in different aqueous extracts

Treatment	Percent egg hatched/day			percent egg hatching inhibition at 9 days
	3	6	9	
Dry Cassava Peel	14.9	27.4	29.9	70.1
Dry Orange Peel	10.4	23.4	25.2	74.8
Fresh Cassava Peel	9.0	19.0	20.9	79.1
Fresh Orange Peel	6.9	11.6	12.2	87.8
Sterilized water (control)	45.8	89.5	98.2	1.8
Lsd (0.05)	2.5	6.5	5.2	5.5
CV (%)	5.6	6.7	5.5	6.6

There were significant differences ($P < 0.05$) between aqueous extract of fresh 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, the percentage egg hatching inhibition at nine days after inoculation showed significant differences ($P < 0.05$) between fresh orange peel aqueous extract and the rest of the treatments (Table 1). The highest percentage of egg hatching inhibition in the fresh orange peel aqueous extract may be attributed to the presence of phytochemicals such as limonene which suppressed egg hatching. According to Isman *et al.* (15) limonene in 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.* (16) reported that citrus waste 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 (17).

2.1.2 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 orange peel had the highest 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 orange peel aqueous extract (35.4, 70.6 and 86.4%) at 12, 48 and 72h, respectively. The lowest mortality was observed in the sterilized water (Table 2).

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

Treatments	Percent mortality/h		
	12	48	72
Dry Cassava Peel	22.0	57.0	62.6
Dry Orange Peel	35.4	70.6	86.4
Fresh Cassava Peel	36.2	71.0	86.6
Fresh 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 orange peel and the rest of the treatments applied (Table 2). Tsai (18) reported that aqueous extract of orange peels caused mortality rate of juveniles by 93.5% after 72h of exposure. The highest mortality rate in the fresh orange peel aqueous extract could be due to higher stability of lemonene in water compared to hydrogen cyanide. Li and Chang (19) stated that lemonene moderately dissolve in water and remained stable for eight weeks after storage, while hydrogen cyanide had low persistence in water.

Comparing the fresh peels and dry peels, it was observed that fresh peels of 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. Studies have shown that concentration of lemonene is higher in the fresh peels compared to the dry peels. Kamal *et al.* (20) reported that 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 %).

2.2 Experiment 2. Results and discussion

2.3 Effect of sweet orange and cassava peels and their combinations on root-knot nematodes in the field

2.3.1 Effect of organic amendments on the yield of tomato in the major and minor seasons

The highest yield of tomato fruits was recorded in the combined application of fresh orange and cassava peels, while the lowest yield was obtained in the no application (control) (Table 3). There were significant differences ($P<0.05$) between the combined application of fresh orange and cassava peels and the rest of the treatments except the combined application of fresh cassava and orange peels (Table 3). The highest yield observed in the combined application of fresh orange and cassava peels can be explained by the high number of colony of *trichoderma* sp and

low root-knot nematode population. The level of reduction in quantity and/or quality of yield of crops due to root-knot nematode damage is positively correlated to their population (21).

Table 3 Effect of treatments on fruit yield of tomato in the major and minor rainy seasons.

Treatments	Yield kg/ha	
	major season	minor season
Fresh Orange Peel (FOP)	19212.0	15289.0
Dry Orange Peel (DOP)	18172.0	14373.0
Fresh Cassava Peel (FCP)	17508.0	14660.0
Dry Cassava Peel (DCP)	17476.0	12394.0
FCP +FOP	20774.0	16170.0
FOP + FCP	20981.0	16707.0
DOP +DCP	18832.0	14057.0
DCP + DOP	18547.0	14008.0
NPK Only	15926.0	9943.0
Carbofuran	19066.0	15155.0
No application (control)	6512.0	3311.0
Lsd (0.05)	834.6	662.2
CV %	3.2	3.5

The yield observed under the different test treatments had consistent trend over the major and minor seasons. However, significant yield reduction was recorded in the minor season across the treatments. The drop in yield for all the treatments in the minor season might be attributed to massive flower abortion due to the sensitivity of the variety to cold weather condition. During this period, the average temperature recorded at the trial site was 29.6°C Maximum and 20.8°C Minimum. According to Khodorova and Boitel-Conti (22) extreme temperature such as high

daytime temperatures (above 29° C), or high nighttime temperatures (above 21°C), or low nighttime temperatures (below 13°C) can cause significant flower dropping in tomato.

2.3.2. Number of juveniles extracted from the roots and soil of tomato in the major season

The number of root-knot nematode eggs, number of juveniles extracted from the roots and number of root-knot nematode adults from the soil of tomato grown in the major season is presented in Table 4. The highest percentage reduction of root-knot nematode eggs (47.6%), juveniles in the roots (69.3%) and adults in the soil (75.9%) over the control was observed where carbofuran was applied, followed by the combined application of fresh orange and cassava peels (42.6, 63.4 and 74.0%). The application of NPK fertilizer alone had the lowest percentage reduction of root-knot nematode eggs (0.9%), juveniles from the roots (9.8%) and adults from the soil (10.0%) over the control (Table 4). The carbofuran was significantly different ($P < 0.05$) from the rest of the treatments except the combined application of fresh orange and cassava peels (Table 4).

410 **Table 4 Gall index, number of eggs, number of juveniles in the root and number of nematodes in the soil of tomato in the**
 411 **major season**

Treatments	Root-knot nematode Gall index	Root-knot nematodes eggs/10g root	% Root- knot nematodes reduction of eggs	Root-knot nematode juveniles/10g root	% Root- knot nematode reduction from roots	Root-knot nematode/100ml soil	% Root- knot nematode reduction from soil
Fresh Orange Peel (FOP)	2.5	1061.0	30.2	117.0	42.9	379.0	64.8
Dry Orange Peel (DOP)	2.5	1183.0	22.2	131.0	36.1	408.0	62.2
Fresh Cassava Peel (FCP)	2.8	1278.0	16.0	128.0	37.6	432.0	59.9
Dry Cassava Peel (DCP)	3.8	1388.0	8.7	129.0	37.1	475.0	55.9
FCP + FOP	1.8	873.0	42.6	75.0	63.4	329.0	69.5
FOP + FCP	1.3	816.0	46.4	65.0	68.3	280.0	74.0
DOP +DCP	2.5	1124.0	26.1	130.0	36.6	383.0	64.5
DCP + DOP	2.5	1169.0	23.1	132.0	35.6	395.0	63.4
NPK fertilizer	5.0	1507.0	0.9	185.0	9.8	970.0	10.0
Carbofuran	1.3	797.0	47.6	63.0	69.3	260.0	75.9
Non application (control)	6.3	1521.0	-	205.0	-	1078.0	-
Lsd (0.05)	0.4	81.2	-	16.0	-	81.1	-
CV(%)	8.1	2.4	-	3.9	-	5.5	-

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

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

415 % percentage values were calculated over the control

The combined application of fresh orange and cassava peels was found to be as good as carbofuran in controlling root-knot nematode (Table 4). This might be due to the combined effect in the application of fresh orange peels first followed by the application of fresh cassava peels. The application of fresh 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 agree with that of Sundararaju and Kumar (23) who reported that the integration of organic amendment and green manure was more effective in reducing the number of nematodes and subsequently increasing plant growth and yield than the organic treatment alone.

2.3.3. Population of root-knot nematode extracted from the root and soil of tomato plants in the minor season

The combined application of fresh orange and cassava peels had significantly reduced gall index (1.3) and number of eggs of root-knot nematodes (61.4%) as good as carbofuran over the control (Table 5). Significant differences ($P < 0.05$) occurred between the combined application of fresh orange and cassava peels and the rest of the treatments tested except the combined application of fresh cassava and orange peels and carbofuran. Osei *et al.* (16) indicated that citrus waste inhibited egg hatching by more than 90% after 72 hours of exposure *in vitro*. The reduction of the number of root-knot nematode eggs on the roots have corresponding effect on their population in the root and soil and gall index (Table 5). Therefore, the reduction in the gall index due to the combined application of fresh orange and cassava peels was partly as a result of low number of eggs. According to Jaiteh *et al.* (24) the highest plant inoculation level of root-knot nematodes eggs on tomato resulted in the highest number of eggs, juveniles and fresh root weight.

449 **Table 5. Initial root-knot nematode population, gall index, number of egg and number of root knot nematodes extracted from**
 450 **the root and soil of tomato at the field in the minor season**

Treatments	Root-knot nematode gall index	Root-knot nematode eggs/10g root	% Root-knot nematode egg reduction	Root-knot nematode juveniles/10g root	% Root-knot nematode juveniles reduction	Root-knot nematodes/100ml soil	% Root-knot nematode reduction from soil
Fresh Orange Peel (FOP)	1.9	831.0	52.2	108.0	52.2	278.0	75.0
Dry Orange Peel (DOP)	2.3	949.0	45.4	133.0	41.2	343.0	69.2
Fresh Cassava Peel (FCP)	2.8	969.0	44.2	136.0	39.8	365.0	67.2
Dry Cassava Peel (DCP)	3.0	1178.0	32.2	143.0	36.7	513.0	53.9
FCP + FOP	1.0	709.0	59.2	99.0	56.2	235.0	78.9
FOP + FCP	1.0	670.0	61.4	88.0	61.1	190.0	82.9
DOP +DCP	2.3	852.0	51.0	127.0	43.8	313.0	71.9
DCP + DOP	2.3	880.0	49.3	132.0	41.6	324.0	70.9
NPK fertilizer	7.0	1725.0	0.7	218.0	3.5	1018.0	8.5
Carbofuran	1.0	635.0	63.4	85.0	62.4	175.0	84.3
No application (control)	7.3	1737.0	-	226.0	-	1113.0	-
Lsd (0.05)	0.5	48.6	-	10.8	-	44.9	-
CV (%)	5.6	3.2	-	3.0	-	3.2	-

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

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

453 % percentage values were calculated over the control;

Highest percentage reduction of juveniles in the roots (62.4%) and soil (84.3%) was observed in plot treated with carbofuran, followed by the combined application of fresh orange and cassava peels (61.1 and 82.9%), while the lowest reduction of juveniles in the root (3.5%) and soil (8.5%), respectively, over the control treatment was observed in the NPK fertilizer treated plots (Table 5). Significant differences ($P < 0.05$) were observed between carbofuran treated plots and the rest of the treatments except the combined application of fresh orange and cassava peels (Table 5). The potential of the fresh orange and cassava peels combination in reducing the population of root-knot nematodes in the roots and soil of tomato as good as carbofuran could be due to the combined effect and higher presence of *Trichoderma* sp. The colony count of *Trichoderma* sp was observed to be higher in the combined application of fresh orange and cassava peels than all the other treatments tested (Table 6). Previous research has shown that *Trichoderma* spp have good potentials for plant parasitic nematode control. Khan *et al.* (25) 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.

2.3.4. Effect of the organic amendments on *Trichoderma* sp and free living nematodes in the soil

The types of microorganisms predominantly found in the soil were *Trichoderma* sp as nematophagus fungus, *Heterocephalobellus* spp and *Eucephalobus* spp as bacterivorous nematodes and *Dictylenchus* spp as fungivorous nematode (Table 6). The combined application of fresh orange and cassava peels significantly increased number of colonies of *Trichoderma* sp (9 colonies), followed by the application of fresh orange peel alone (8 colonies). The lowest number of colonies was observed in the application of carbofuran (0 colony) (Table 6). The high colony count in the combined application fresh orange and cassava peels might be due to the presence of certain chemical compounds that favours growth and multiplication of *Trichoderma* sp compared to the other treatments (cassava peels, NPK alone and carbofuran). According to Duli, *et al.* (26) results obtained from laboratory experiment on the evaluation of different substrates for *T. harzianum* showed highest growth rate and sporulation on orange peel substrate compared to other substrates tested.

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

Treatments	No. of fungus and free living nematodes							
	Nematophagus fungus		Bacterivorous nematodes				Fungivorous nematode	
	<i>Trichoderma</i> sp		<i>Heterocephalobellus</i> sp		<i>Eucephalobus</i> sp		<i>Ditylenchus</i> sp	
	Before application	After application	Before application	After application	Before application	After application	Before application	After application
Fresh Orange Peel (FOP)	1	8	3	1	2	1	3	2
Dry Orange Peel (DOP)	1	6	3	0	4	1	2	1
Fresh Cassava Peel (FCP)	1	2	4	24	3	18	2	23
Dry Cassava Peel (DCP)	1	1	2	17	2	16	3	25
FCP +FOP	1	8	1	8	1	7	1	5
FOP + FCP	1	9	0	2	1	2	0	3
DOP + DCP	2	7	0	1	1	1	2	2
DCP + DOP	1	6	1	5	2	4	2	6
NPK fertilizer	2	2	0	4	1	3	2	2
Carbofuran	1	0	1	0	3	1	1	0
No application (control)	2	1	1	3	2	4	1	3

487

488
 489 In the case of bacterivorous and fungivorous nematodes, the highest count was observed in
 490 application of fresh and dry cassava peels compared with fresh and dry orange peels and their
 491 combinations (Table 6). The bacterivorous and fungivorous nematodes extracted from the soil
 492 after application of the treatments were found to be significantly lower in the application of
 493 carbofuran and plots treated with fresh and dry orange peels (Table 6). Odeyemi *et al.* (27)
 494 reported that addition of organic amendments in the soil had resulted in population increase of
 495 free-living nematodes. Potential changes in the abundance and community structure of free-
 496 living nematodes was observed following the application of organic amendments in the surveyed
 497 areas (28). Although, the cassava peel contain cyanide in the peels which kills nematode (5)
 498 however, cyanide is easily degraded under high temperature and in water (19). This low
 499 persistence of cyanide in cassava peels might have favoured the rapid multiplication of the
 500 bacterivorous and fungivorous nematodes at where fresh and dry cassava peel were applied.

501 502 **Conclusion**

503
 504 At the laboratory, higher percentage of egg hatch inhibition and mortality of root-knot nematode
 505 juveniles was observed in the fresh orange peel aqueous extract compared with the rest of the
 506 treatments. The fresh orange and cassava peels had higher percentage egg hatch inhibition and
 507 mortality of root-knot nematodes than the dry orange and cassava peels. The field experiment
 508 carried out in the two first seasons (major and minor season) showed no significant difference
 509 ($P < 0.05$) between carbofuran and the combined application of fresh orange and cassava peels in
 510 all the nematode parameters observed. However, the combined application of fresh orange and
 511 cassava peels significantly increased the yield of tomato more than the application of carbofuran
 512 in both seasons. Higher number of colony of *trichoderma* sp was observed in the combined
 513 application of fresh orange and cassava peels compared with the other treatments tested. The
 514 fresh peels of orange and cassava were observed to significant reduce gall index, number of root-
 515 knot nematode eggs, number of root-knot nematode juveniles in the root and number of
 516 nematodes in the soil more than the dry orange and cassava peels.
 517 The bacterivorous and fungivorous nematodes were found to be higher in the application of fresh
 518 and dry cassava peels more than the other treatments. The study recommended for multi-location
 519 testing of the combined application of fresh 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.

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