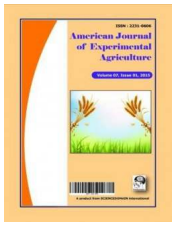

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Efficacy of Sweet Orange and Cassava Peel Amendments for the Management of Root-knot Nematodes on Tomato

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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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 December, 2015.
Methodology: Five aqueous extracts of: 1) Fresh sweet orange peel (FOP), 2) Fresh cassava peel (FCP), 3) Dry sweet orange peel (DOP) and 4) Dry cassava peel (DCP) on egg hatching inhibition and juvenile mortality of root-knot nematodes were studied at the laboratory. Sterilized water was used as the control. Eleven treatments: 1) FOP, 2) DOP, 3) FCP, 4) DCP, 5) FCP+FOP, 6)

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FOP+FCP, 7) DOP+DCP, 8) DCP+DOP, 9) NPK and ($(NH_4)_2$ SO_4) fertilizers, 10) Carbofuran and 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 in the FOP aqueous extract. Significant differences ($P<0.05$) were observed between FOP aqueous extract and the rest of the treatments in the mortality of root-knot nematodes. For the field experiment, combined application of FOP and FCP significantly increased ($P<0.05$) yield more than the rest of the treatments. Carbofuran, followed by the combined application of FOP and FCP significantly reduced ($P<0.05$) gall index, number of eggs, number of root-knot nematode juveniles in the root and the soil in both seasons. However, no significant difference ($P>0.05$) was observed between carbofuran and combined application of FOP and FCP.

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 extracts of FOP, DOP and FCP showed high potential in egg hatching inhibition and juvenile mortality of root-knot nematodes. Further evaluation of the combined application of FOP and FCP 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 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 [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 health and environmental 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 [4-6]. Neem seed cake, castor seed cake and castor bean aqueous extract, have been widely studied for their nematicidal properties [4,7].

Sweet orange and cassava are largely consumed in Ghana but most of the peels end up as wastes [8], 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. Loumédjinon et al. [9] 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 conducted on fresh 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 [10]. Farmers mostly depend on cultural practices such as crop rotation, which sometimes 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 biocontrol 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 and their effect on fruit yield. Also, to evaluate the effect of sweet orange

and cassava peels on the 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*

It was necessary to identify the nematicidal potential of sweet orange and cassava peels for the investigation of the root-knot management. The experiment was arranged in Complete Randomized Design (CRD) with five replications made up of five treatments.

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 was 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 0.5 cm width and 0.5 cm length. Portions of the fresh peels of sweet orange and cassava were air-dried under shade in the shade house for two weeks to obtain the dry peels. For standardization, a sub-sample of 100 g each of fresh and dry peels of sweet orange and cassava were oven-dried separately at 70℃ for 72 h and then weighed to determine the dry matter content [9]. The equivalent to 50 g dry matter of each type of peel was soaked in 150 ml of distilled water (1:3 w/v dilution) for 24 h in a beaker at 28℃. The aqueous extract of each peel type was collected by sieving through cheesecloth. Twenty millilitres (20 ml) of each aqueous extract was dispensed into separate 9cm 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℃ for 3 h. The soil mixture was moistened with water before placing in the barrel. After steam-sterilization, the soil was allowed to cool 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 8 kg of the steam-sterilized soil mixture and 21-day-old tomato seedlings planted in sterilized top-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 shade house. 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 0.5 cm length with a pair of scissors. The root-knot nematodes juveniles were extracted using modified Baermann tray method [11]. Extraction of root-knot nematode eggs was done using modified Hussey and Barker method [12].

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 were placed in each Petri dish containing the 20 ml of the aqueous extract from each treatment. The number of dead root-knot nematodes was counted at 24, 48 and 72 h 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 about 28℃ for another 24 h. Nematodes were probed with a sharp-edge needle under a stereo microscope and the ones that were straight in shape and remained immobile even after probing were considered dead [13].

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 20 ml of each aqueous extract. The Petri dishes were placed on a laboratory bench at about 28℃ 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 region 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 1800 mm. The soil was loamy sand Dystric Cambisol with a composition of 87.3% sand, 7.0% silt and 5.7% clay in the topsoil (0-15 cm). 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* (Linn.) 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 1 m between plots and blocks. The experiment was repeated for two seasons at the same location; the first experiment was carried out during the major season (June to September, 2014) and the second during the minor season (November, 2014 to January, 2015). The experiment was carried out under natural conditions without any nematode inoculation.

The experiment consisted of the following treatments:

1) 50 g/plant of fresh sweet orange peel
2) 50 g/plant of dry sweet orange peel
3) 50 g/plant of fresh cassava peel
4) 50 g/plant of dry cassava peel
5) Zero application (control)
6) 25 g/plant of fresh cassava peel + 25 g/plant of fresh sweet orange peel
7) 25 g/plant of fresh sweet orange peel + 25 g/plant of fresh cassava peel
8) 25 g/plant of dry sweet orange peel + 25 g/plant of dry cassava peel
9) 25 g/plant of dry cassava peel + 25 g/plant of dry sweet orange peel
10) NPK (15:15:15) + Ammonium sulphate ($(NH_4)_2\ SO_4$) 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 using a hoe to the height of about 20 cm. 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 0.5 length and 0.5 cm width). The weight of fresh and dry peels was determined based on dry matter content as described above. Each type of peel was applied at the rate of 50 g dry matter/plant [9]. The single dose treatments were applied four weeks before seedlings were transplanted. The peels were placed on the ridges in 10 cm diameter circle at 40 cm apart and thoroughly incorporated into the

soil with a hand fork to a depth of 10 cm. The first part (25 g) of the combined treatments was applied four weeks before and the remaining part (25 g) was 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 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 0.5 g per plant and incorporated as the organic amendment described above. The product was manufactured by Shenzhen Baocheng Chemical Industry, China.

2.2.5 Seedling preparation, transplanting of seedlings and application of fertilizers

The seedlings were grown in boxes filled with steam-sterilized top-soil. Seeds were sown in lines at a depth of 2 cm and 15 cm apart. Four-week-old tomato seedlings (var. Power) were transplanted onto the ridges at a spacing of 50cm intra-row and 90 cm 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) 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 [$(NH_4)_2$ SO_4] was applied at a rate of 125 kg/ha [14] at six weeks after transplanting. Both fertilizers (NPK and $(NH_4)_2$ SO_4) were applied in rings at 5 cm radius and 10cm deep from the base of each plant and covered with soil.

2.2.6 Application of fungicides and insecticides

Merpan® 50 WP (Captan 500 g/kg) and Folpan® 50 WP (Folpet 500 g/kg) fungicides were applied at the rate of 1.2 kg/ha every two weeks. The insecticide Acceta Star® (16 g Acetamiprid/L and 30 g Bifenthrin/L) was applied every week at the rate of 1L per hectare. Golan® (200 g of Acetamiprid/L) was also applied at the rate of 0.5 L/ha every two weeks to control whiteflies. Both chemicals were manufactured by Makhteshim Chemical Works Ltd in Israel. The 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 was carried out twice daily when there was no rainfall during the minor season. In order to avoid erosion of the materials, water was sprinkled on the soil using watering cans.

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

The tomato plant roots were uprooted 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 as described above. Ten grams 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 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 [11]. The modified Hussey and Barker method [12] was used to extract the eggs from the roots.

2.2.9 Morphological identification of nematodes

Nematodes relaxed and preserved in formalin and glycerol [15] 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 images of the nematodes were recorded at magnifications 25x and 40x and were later used for identification using manuals [16,17]. 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.

2.2.10 Analysis of nutrients and cyanide in orange and cassava peels

Samples of sweet orange and cassava peels were collected and taken to Soil science laboratory of the Department of Crop and Soil Sciences, KNUST for nutrient analysis. The samples were oven-dried at 70℃ for three days before processing. Each sample was ground into powder using a grinding machine (Micro doughLAB, Perten Instrument Group, Canada) to carry out the following analysis:

1. Organic carbon (%)
2. Total Nitrogen,
3. Potassium and
4. Available Phosphorus

The organic carbon content was determined using Walkley-Black Method [18] and Bray's method No 1 [19] was used for potassium and available Phosphorus analysis. The total cyanide was determined in fresh and dry cassava peels using Cooke method [20].

2.2.11 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.

2.2.11.1 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. The yield data collected at different intervals were summed up to have the final data for each treatment.

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

Galling was assessed on the roots of tomato plants after harvest using gall index scale of 0-10 (21) 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.

2.2.11.3 Extraction of fungi and free-living nematodes from 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 in the laboratory for 48h before isolation. Fungi were isolated by serial dilution [22] 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 (Leica, Leica Microsystems Company Ltd., Germany), specimen and reference manuals [22]. Part of the same samples was used to extract and determine the population of free-living nematodes. The free-living nematodes were extracted from the soil using Baermann tray method [11].

2.12 Data Analyses

The data collected from the experiments were subjected to ANOVA using GenStat 12th edition software. The means were separated using Tukey test at $P<0.05$. The nematode and egg counts were square root transformed $\sqrt{(x+1)}$, where x is the mean count before statistical analysis and back-transformed. The transformation was done because the data tested did not follow normal distribution. Correlation analysis was done to find out the relationship between *Trichoderma virens* and root-nematode population in the soil.

3. RESULTS AND DISCUSSION

3.1 Laboratory Experiment: Evaluation of Sweet Orange and Cassava Peel Aqueous Extracts for their Nematicidal Potentials on Root-knot Nematodes *in vitro*

3.1.1 Concentration of the chemical properties in the orange and cassava peels

The orange peel had the highest percentage of organic carbon, total nitrogen, potassium and available phosphorus compared to cassava peels (Table 1). For the hydrogen cyanide content in fresh and dry cassava peels, the

highest concentration was observed in the fresh cassava peel compared to dry peel. The concentration of hydrogen cyanide was lower by 50% when the fresh cassava peel was dried under shade for two weeks (Table 1).

3.1.2 Root knot nematode 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 2. There were significant interaction effects ($P<0.05$) between the aqueous extract of sweet orange and cassava peels and observation times at days after inoculation on the egg hatching inhibition of root-knot nematodes. Significant differences ($P<0.05$) occurred between aqueous extract of FOP and the rest of the aqueous extracts except FCP aqueous extract at six and nine days after inoculation (Table 2). 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 2). No significant differences ($P>0.05$) were observed between aqueous extract of FOP and FCP, DOP and DCP at 3 days after inoculation (Table 2).

The highest percentage of egg hatch inhibition in the FOP aqueous extract may be attributed to the presence of phytochemicals such as limonene which suppressed egg hatching. According to Isman et al. [23] 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. [24] reported that citrus peels inhibited egg hatching by more than 90% after 72h of exposure *in vitro*. The FOP and FCP aqueous extracts significantly inhibited egg hatching more than DOP and DCP aqueous extracts in all the observation periods. This could be due to the high presence of the nematicidal properties such as cyanide in the FCP than DCP [25] (Table 1). All the peels studied significantly inhibited the hatching of nematode eggs when compared to control over time. 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. [26], evolution in the eggshell structure of *Meloidogyne* spp. before hatching 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 [26].

3.1.3 Mortality of root-knot nematode juveniles in the different aqueous extracts *in vitro*

Table 3 shows the effect of aqueous extracts of the organic amendments on the mortality of root-knot nematode juveniles at 12, 48 and 72 h after application. Significant interaction effects ($P<0.05$) were observed between the aqueous extracts of the various peels and periods after inoculation on the mortality of root-knot nematode juveniles.

The result of the percentage mortality followed a similar trend in all the observation periods. The aqueous extract of FOP had significantly higher ($P<0.05$) nematode mortality in all the three periods observed (45.6, 86.6 and 94.8%), followed by FCP aqueous extract (36.2, 71.0 and 86.6%) and DOP aqueous extract (35.4, 70.6 and 86.4%) at 12, 48 and 72h respectively (Table 3). Similar result was observed by Tsai [27] who reported that aqueous extract of sweet orange peels caused mortality rate of root-knot nematode juveniles by 93.5% after 72 h of exposure. The highest mortality rate in the FOP aqueous extract could be due to higher stability of lemonene in water compared to hydrogen cyanide. Li and Chang [28] 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. [29] reported that lemonene, which is one of the major constituents in sweet orange peel essential oil exhibited strong nematicidal activity against *M. incognita*.

The mortality rate had significantly increased ($P<0.05$) in both sweet orange and cassava aqueous extracts over time. However, the rate of increase of mortality was significantly higher ($P<0.05$) in the aqueous extract of FOP than the rest of the treatments, followed by FCP aqueous extract (Table 3). Andre et al. [30] reported an increase in the rate of mortality of root-knot nematodes in the essential oil of *Citrus sinensis* with time after inoculation. The high mortality rates of root-knot nematode juveniles in the FOP and FCP aqueous extracts might be attributed to the higher presence of the nematicidal properties (limonene and cyanide) in the fresh peels

compared to the dry peels [25,30]. The laboratory analysis of cyanide contents in fresh and dry cassava peel showed higher content (more than 50%) in the fresh peels than dry peels (Table 1).

3.2 Field Experiment: Effect of Sweet Orange and Cassava Peels and their Combinations on Root-knot Nematodes of Tomato in the Field

The mean initial population of 686 juveniles of root-knot nematodes/100 ml 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 the major and minor seasons are presented in Table 4. The gall indices and number of root-knot nematode eggs were not significantly different ($P>0.05$) between the application of carbofuran and combined application of FOP and FCP in both seasons. 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 FOP and FCP compared with the other treatments in the major and minor seasons (Table 4). Similarly, no significant differences ($P>0.05$) were observed between the application of FOP alone and combined application of DOP and DCP (Table 4). The significant reduction of gall index and number of eggs in the combined application of FOP and FCP 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* [31]. Higher mortality of root-knot nematodes and reduction in root galling were observed after incorporation of cassava peels into the soil planted with pepper [32]. In addition, low galling and number of eggs on the roots could be associated with the potential of the combined application of FOP and FCP in reducing the population (Table 4). Hussain et al. [33] 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.

The number of root-knot nematode juveniles in the roots and soil were also significantly reduced ($P<0.05$) in the carbofuran and FOP and FCP combination treated plots compared with the other treatments. However, carbofuran was not significantly different ($P>0.05$) from the combined application of FOP and FCP in both seasons (Table 4). Incorporation of dry citrus peels in the soil highly suppressed plant parasitic nematodes in pineapple production [31]. Similarly, Loumédjinon et al. [9] indicated that dry sweet orange and cassava peels significantly reduced the number of root-knot nematodes in the soil and roots of carrot compared to control. Also, John et al. [34] reported significant reduction of second stage juveniles of *M.incognita* in the soil treated with dry cassava peels compared to untreated plots. Generally, combined application of FOP and FCP performed better than the sole application of the peels in all the parameters measured in the major and minor seasons. This result agrees with that of Sundararaju and Kumar [35] 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.

Table 1. Chemical properties of the orange and cassava peels used for root-knot nematodes management

Organic amendments	% Organic carbon	% Total nitrogen	Potassium (K) cmo/kg	Avail. phosphorus (P) mg/kg	Total hydrogen cyanide (mg/kg)
Orange peel	63.1	1.2	3.8	0.1	0.0
Cassava peel	56.66	0.8	2.6	0.1	15.0 (33.0*)

Values for fresh cassava peel

3.2.2 Effect 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 5. The *C. gloeosporioide* and *A. niger* were found to be the most prevalent fungi, while the *Heterocephalobellus* sp.and *Ditylenchus* sp. were the most abundant bacterivorous and fungivorous nematodes respectively before the application of the treatments (Table 5).

The types of microorganisms predominantly found in the soil were *Trichoderma virens* as nematode biocontrol fungus, other fungi (*C. gloeosporioides*, *P. chrysogenum*. and *A. niger*), *Hetetocephalobellus* sp. and *Eucephalobus* sp. as bacterivorous nematodes and *Dictylenchus* sp. as fungivorous nematode after application of treatments (Table 6). The combined application of FOP and FCP significantly increased ($P<0.05$) number of colonies of *T. virens* (9 colonies), followed by the application of FOP alone (8 colonies) (Table 6). This might be due to the presence of certain chemical compounds that favours growth and multiplication of *T. virens* compared to the other treatments (cassava peels, NPK (15:15:15) and $((NH_4)_2\ SO_4)$ fertilizers and carbofuran). According to Duli et al. [36], 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 *T. virens*, have good potentials in controlling plant parasitic nematodes. Khan et al. [37] 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. This must have contributed to the low population and damage of root-knot nematode observed in the sweet orange peels amended plots in the minor season (Table 4).

Table 2. Eggs hatch inhibition at 3, 6 and 9 days after inoculation in different aqueous extracts

Treatment	Percent egg hatch inhibition/ days after inoculation		
	3	6	9
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
Sterilized water (control)	54.2 c	10.5 d	1.9 d
Treatments (P-value)	0.001		
Observations (P-value)	0.001		
Treatments x Observations (P-value)	0.001		

**Means followed by different letters in the column are significantly different according to Tukey Test at P<0.05*

Table 3. Mortality of root-knot nematode juveniles in different aqueous extracts *in vitro*

Treatment	Percent mortality/hours after inoculation		
	12	48	72
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	76.6 a	94.8 a
Sterilized water (control)	0.0 d	0.0 d	0.0 d
Treatments (P-value)	0.001		
Observations (P-value)	0.001		
Treatments x observations (P-value)	0.001		

**Means followed by different letters in the column are significantly different according to Tukey Test at P<0.05*

Table 4. Gall index, number of egg and juveniles of root-knot nematodes extracted from the root and soil of tomato at harvest in the major and minor seasons

Treatments	[x] Gall index /season		[z] No. of eggs/10 g root /season		[z] No. of Juveniles/10 g root/season		[z] No. nematodes/100 ml soil/season	
	Major	Minor	Major	Minor	Major	Minor	Major	Minor
Fresh Sweet orange Peel (FOP)	3d*	2d	1749de	1580de	398d	248de	758de	542ef
Dry Sweet orange Peel (DOP)	3d	2d	2119cd	1903cd	436d	354d	858d	675d
Fresh Cassava Peel (FCP)	3d	3c	2206cd	2047cd	456cd	366d	880d	742d
Dry Cassava Peel (DCP)	4c	5b	2678bc	3378b	570c	847c	1217c	1633c
FCP +FOP	2e	1e	1667e	1473def	269e	216e	693ef	425fg
FOP + FCP	1f	1e	1576e	1203ef	254e	202e	574f	325g
DOP + DCP	3d	2d	2051de	1610cde	401d	311d	775de	633de
DCP + DOP	3d	2d	2072de	1756cd	412d	343d	837d	638de
NPK (15:15:15) and $((NH_4)_2 SO_4)$ fertilizers	5b	7a	3032ab	4049a	877b	1166b	2139b	3025b
Carbofuran	1f	1e	1840de	1086f	209e	143e	616f	327g
No application (control)	6a	7a	3225a	4774a	1095a	1344a	2308a	3350a
Treatments (P-value)	0.001		0.001		0.001		0.001	
Seasons (P-value)	0.933		0.455		0.426		0.013	
Treatment x Season (P-value)	0.001		0.001		0.001		0.001	

**Means followed by different letters in the column are significantly different according to Tukey Test at P<0.05*

z All count data were back-transformed after transformation ; x Galling on scale 0-10, where 0 = no galls and 10 = root system completely galled

Table 5. Fungi and free-living nematodes isolated from the composite soil sample before application of treatments

No. of fungi and free-living nematodes						
No. of fungal colonies/1 g soil				No. of bacterivorous nematodes/100 ml soil		No. of fungivorous nematodes/100 ml soil
Trichoderma virens	*Colletptrichum gloeosporioides*	*Penecillium chrysogenum*	*Aspergillus niger*	*Heterocephalobellus* sp.	*Eucephalobus* sp.	*Ditylenchus* sp.
2	4	3	4	36	32	44

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

Treatment	Number of fungal colonies/1 g soil after treatment				Number of bacterivorous/100 ml soil after treatment		Number of fungivorous/100ml soil after treatment
	Trichoderma virens	***Colletotrchum gloeosporioides***	***Penicillium* sp.**	***Aspergillus niger***	***Heterocephalobellus* 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 (15:15:15) and $((NH_4)_2\ SO_4)$ fertilizers	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
P-value	0.002	0.001	0.001	0.002	0.001	0.001	0.001

**Means followed by different letters in the column are significantly different according to Tukey Test at P<0.05*

The populations of bacterivorous and fungivorous nematodes in carbofuran, FOP and DOP treated soils were significantly lower (P<0.05) compared to untreated soils (Table 6). However, the application of FCP and DCP significantly increased (P<0.05) the number of bacterivorous and fungivorous nematodes compared with FOP and DOP. Odeyemi et al. [38] reported that addition of organic amendments in the soil 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 [39]. Although, the cassava peel contains cyanide which kills nematode [9] however, cyanide is easily degraded under high temperature and in water [40]. 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 were 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 FOP and FCP than the rest of the treatments except FCP and FOP in the major and minor seasons (Table 7).

The highest yield observed in the combined application of FOP and FCP can be explained by the low galling and root-knot nematode population (Tables 4). 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 of yield of tomato in the combined application of FOP and FCP compared to carbofuran (Table 1). Application of cassava peels increased yield of maize [41]. Also, 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 [42]. Furthermore, the high presence of *T. virens* in the combined application of FOP and FCP might have helped to significantly reduce the root-knot nematode population density and damage and correspondingly increased yield of tomato in the field. The correlation analysis between the population densities of *T. virens* and root-knot nematodes in the soil was highly significant (P<0.05) but negatively correlated (r= -0.50). The application of *T. harzianum* in neem cake amended-soil was highly effective against *M. incognita* and resulted in better plant status of tomato Kumar and Khanna [43].

The treatment effects on yield had consistent trend in both major and minor seasons (Table 7). 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 tomato variety to harsh weather condition. During this period, the average

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

Treatments	Yield (kg/ha)	
	Major season	Minor season
Fresh sweet orange peel (FOP)	19212b	15289 b
Dry sweet orange peel (DOP)	18172 cd	14373 c
Fresh cassava peel (FCP)	17508 d	14660bc
Dry cassava peel (DCP)	17476 d	12394 d
FCP +FOP	20774 a	16170 a
FOP + FCP	20981 a	16707 a
DOP +DCP	18832 bc	14057 c
DCP + DOP	18547 c	14008 c
NPK (15:15:15) +$((NH_4)_2 SO_4)$ fertilizers	15926 e	9943 e
Carbofuran	19066 b	15155 b
No application (control)	6512 f	3311 f
Treatments (P-value)	0.001	
Seasons (P-value)	0.001	
Treatments x seasons (P-value)	0.001	

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

temperature recorded at the trial site was 29.6℃ Maximum and 20.8℃ Minimum. According to Khodorova and Boitel-Conti [44], extreme temperature such as high daytime temperatures (above 29℃), or high nighttime temperatures (above 21℃), or low nighttime temperatures (below 13℃) can cause significant flower dropping in tomato.

4. CONCLUSION

Application of sweet orange and cassava peels were effective in the management of root-knot nematodes on tomato. Percentage egg hatch inhibition and mortality occurred upon application of sweet orange and cassava peels. Also, galling, number of root-knot nematode eggs and juveniles in the roots of tomato and soil were highly reduced when soil was amended with sweet orange and cassava peels. Application of sweet orange peels enhanced the population of *T. virens*, a biocontrol fungus, while cassava peels enhanced the population of bacterivorous and fungivorous nematodes. The study is recommended for multi-location testing of the combined application of FOP and FCP at various tomato farms. Also, different methods of application such as application in the form aqueous extract should be evaluated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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