



NEMATICIDAL ACTIVITY OF *CARICA PAPAYA* L. SEEDS AND LEAF EXTRACTS ON *MELOIDOGYNE* SPECIES ISOLATED FROM INFECTED *SOLANUM LYCOPERSICUM* L. IN NIGERIA

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ABSTRACT

Active metabolites from plants with nematicidal properties are considered safer to the environment, non-toxic to man than synthetic chemicals for the control of plant parasitic nematodes. An in-vitro nematicidal activity of (*Carica papaya*) seeds and leave extracts on *Meloidogyne* species, infecting *Solanum lycopersicum* L (tomato) was carried out in the nematology laboratory University of Jos, Nigeria. The seed and leave of *Carica papaya* were dried at 26°C, ground into powder and extracted with distilled water. The root-knot nematodes were isolated by Baerman method and identified by comparative morphology, descriptive and lattice keys. The juveniles were exposed to varied concentrations (0.75, 1.5, 3.0 and 6.0 mg/mL) of the extracts incorporated in 1ml of homogenized nematodes suspension (50 juveniles) replicated three (3) times for different times of 6, 12, 18 and 24 hours. Data of immobilized/dead nematodes was recorded for each period. Phytochemical screening of the extract was carried out using standard methods. The data was analyzed using Analysis of variance (ANOVA). The result showed that aqueous seed extract of *Carica papaya* caused 100% nematode juvenile mortality at the highest's concentration (6 mg/mL) within 24 hours of exposure while the leaf extract of *Carica papaya* caused 100% mortality within 24 hours of exposure. The result revealed that the higher the concentration of both seeds and leave extracts of *Carica papaya* the higher the mortality of root-knot juveniles. The effect varied significantly with concentration of the extracts at $P < 0.05$. The bioactive Phyto substances of the test plant revealed the presence of alkaloids, carbohydrates, flavonoids, anthraquinones, tannins, steroids/terpenes, saponins, Cardiac glycosides. This suggests that the extracts contain bioactive substances which were active on *Meloidogyne* species.

Keywords: *Carica papaya*, *Meloidogyne* species, Nematicidal, *Solanum lycopersicum*

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INTRODUCTION

Plant-parasitic nematodes cause significant damage to majority of agricultural crops in tropical and subtropical regions with annual losses estimated to be \$100 billion worldwide. The root-knot nematode (*Meloidogyne* spp) is common and affects a wide range of crops, more than 3000 host species cause serious damage to most agricultural crops around the world [1]. These Plant pathogenic root-knot nematodes reduce crop yield by 8.8 % in developed countries and up to 14.6% in tropical and subtropical regions. They infest a wide range of important crops and are more damaging to vegetables than to others [2].

The tomato (*Solanum lycopersicum*) is the world's most important vegetable crop, but it is frequently attacked by *Meloidogyne* spp, limiting fruit yield quantity and quality [3]. Tomato yield losses due to root-knot nematodes have been estimated to be up to 61.0 %. The root-knot nematodes represent an important constraint on the delivery of global food security.

Damage caused by these nematodes has been estimated at US\$ 80 billion per year [4]. This is likely to be a significant underestimate of the actual figure as many growers in developing nations are unaware of the existence of plant-parasitic nematodes [5]. One of the major obstacles to the production of adequate supplies of food in many developing nations is damage caused by *Meloidogyne* spp. It is generally admitted that four major species, i.e., *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, as well as a few emerging species such as *M. enterolobii* and *M. chitwoodi*, cause the vast majority of crop damage [6].

The control practices have become more challenging because of the banishment of mostly applied nematicides, and the lack of resistant plant material. Antagonistic plants have largely been incorporated into the crop management of root-knot nematodes populations [7 - 9]. Plant derived extracts have long been a subject of research in an effort to develop alternatives to conventional nematicides usually safer and with minimal residual effects [10]. Plants have the ability to synthesize a wide variety of biochemical substances that

are used to perform important biological functions [11]. The use of biological materials for the synthesis of nanoparticles includes plant extracts and seaweeds which avoid the use of toxic chemicals and thus presents a number of advantages over chemical synthesis including Eco friendliness and compatibility for pharmaceutical manufacturing and other biomedical applications [2]. The global nematicide market is expected to continue growing, with an increasing demand for synthetic chemical free organic foods, botanical nematicides are taking the lead as replacements. Consequently, in the recent years there have been vigorous efforts towards identification of the active secondary metabolites from various plants. These include mostly glucocyanates, and their hydrolysis products such as isothiocyanates; flavonoids, saponins and the more recently probed essential oils, among others. And despite their over whelming potential variabilities in quality, efficacy, potency and composition continue to persist, and commercialization of new botanical nematicides is still lagging [12].

In nematode infested cropping systems, control and management is necessary and this involves using integrative approaches [13]. For decades, chemicals like methyl bromide stood as the standard fumigants for managing nematodes. However, these once dominant nematicides are no longer available due to their adverse effects in the environment [14].

Pesticides are generally used for the control of pests and diseases but these chemical pesticides cause human health and environmental hazards. So, there is a need to find alternative methods which are safe and suitable for environment [15]. Plants having nematicidal properties have been used as bio-pesticides [16, 17]. The research aims at using plant extract to test for nematicidal properties on *Meloidogyne* species.

MATERIALS AND METHODS

Study area

The study was carried out in the New Biology Laboratory of the department of Plant Science and Biotechnology, University of Jos. The leaves and seed of *Carica papaya* L. were obtained from Federal College of Forestry and Farin Gada Market in Jos, on latitude 70°22N and longitude 70°22E.

Preparation of plant materials

The seeds and leaves of *Carica papaya* were obtained in clean polythene bags and taken to the laboratory for analysis. The seeds and leaves were dried on laboratory benches under room temperature (26°C) for three (3) weeks. The dried seeds and leaves were pound into powder using pestle and mortar and sieve with fine synthetic mesh into fine powder. Fifty (50 g) grams of each of the powder were weighed and soaked in sterile

distilled water and extracted using cold maceration method of extraction as described by Pagi and Pate [18]. The extracts obtained were used for phytochemical analysis and nematicidal test on root-knot nematodes obtained from infected tomato plants by the Baerman modified method of nematode extraction.

Extraction of *Carica papaya* leaf and seed

The cold maceration method was used for the extraction as described by Pagi and Pate [18]. Fifty grams (50 g) of the powder was weighed with top loading mechanical balance and poured into a conical flask and soaked with the solvent (distilled water). This was allowed to stand for 24hours with periodic shaking. The content was filtered using a muslin clothe and the residue rinsed three times with the extracting solvent. The collective filtrate was filtered through Whatman's filter paper of (20 x 20 size). The filtrate was concentrated to dryness using a water bath and a drying cabinet at a controlled temperature of 45°C. The percentage yield of the extract was determined. The dry extract was then transferred into a sterile sample container and preserved in the refrigerator for further use.

Extraction of nematodes

The root-knot Nematodes were extracted from the roots of tomato plants showing symptoms of galls, after washing the roots free from soil. The extraction of root-knot nematode from infected tomato was carried out by the Baermann modified Funnel method as described by Okechalu *et al.* [12]. The galls from the roots were placed in petri dishes containing a small amount of distilled water to moisten them and then teased apart. The root samples and distilled water in Petri dishes were inverted into funnels with a short piece of rubber tubing attached to the stem. A test tube filled with water was then attached to the end of the rubber tubes and were made air tight at the points of attachment with the funnel and test tube using masking tape, the funnels were then lined up with thin layer of cotton wool and supported in an upright position on the table.

Water was added to the root samples in the funnel to prevent them from drying; thus, allowing free movement of nematodes, the set up was allowed to stand overnight for about 24 hours. Nematodes juveniles that hatched and swam through the cotton wool were collected at the bottom of the test tube. Root-knot nematodes infection is often identified by swelling in roots that look like 'knots or galls that are large and easy to see with naked eye. The microscopic identification was done by used of comparative morphology, descriptive, and lattice keys. Female shapes are oval while male characters are stylet length and shape, stylet cone length, head shape, and distance of esophageal gland outlet from stylet base [19].

In-vitro nematicidal test

The *in-vitro* nematicidal activity of seed and leaf extract of *Carica papaya* on root-knot nematodes juveniles was carried out by exposure to varied concentrations of 0.75, 1.5, 3.0 and 6.0 mg/mL to 1 mL of homogenized suspension of nematodes (50 juveniles). Each was replicated three (3) times and kept at a room temperature. The mixture was examined at intervals of 6, 12, 18 and 24 hours for life and dead nematodes. The mortality was assessed by touching the juveniles with a fine needle to trigger the movement thus, observing whether immobilized or a live [20]. These were counted and recorded. Juvenile nematodes suspension with distilled water was used as control.

Phytochemical analysis

The *Carica papaya* seeds and leaves extracts were screened for their biochemical constituent to determine the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, cardiac glycosides, saponins, steroids/terpenes and tannins using standard phytochemical screening procedure as described by the Association of Official Analytic Chemists (A.O.A.C.) [21]. Five percent (5%) Sulphuric acid and one milliliter (1 mL) of Wagner's reagent was added drop by drop. A reddish-brown precipitate indicated the presence of alkaloids.

Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones, 0.5 g of each extract was taken into a dry test tube and 5 mL of chloroform was added and shook for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones.

Test for carbohydrates (Molish test)

Few drops of alcoholic α -naphthol solution were added to two milliliters (2 mL) of extract. Later, few drops of concentrated H_2SO_4 were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.

Test for cardiac glycosides

100 mg of the extract was dissolved in 70% alcohol and filtered. About 3 drops of lead sub-acetate was

introduced into the filtrate and filtered. The filtrate was extracted with 10 mL of chloroform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1 mL of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1 mL of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxy sugar characteristic of cardenolides.

Tests for flavonoids (Alkaline reagent test)

Two to three drops of sodium hydroxide were added to two milliliters (2 mL) of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCl, indicating that flavonoids were present.

Tests for saponins

A drop of Na_2CO_3 solution was added to five milliliters (5 mL) of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

Test for steroids/terpenes

Two milliliters (2 mL) of the extracts was mixed with one milliliter (1 mL) of acetic anhydride and one milliliter (1 mL) of concentrated sulphuric acid was carefully added down the side of the test tube. The appearance of a brownish-red color indicated the presence of steroids.

Test for tannins (Ferric chloride) test

Five milliliters (5 mL) of distilled water was added to two milliliters (2 mL) of the extract and three (3) drops of ferric chloride was added to the extract. The mixture was observed for a blue or greenish-black color which indicated the presence of tannins.

Statistical analysis

Data collected were analyzed using analysis of Variance (ANOVA) at 5% level of probability, where significance means were separated using Least Significant Difference (LSD).

RESULTS

The result of the phytochemical screening of aqueous seed and leaf extract of *Carica papaya* showed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, anthraquinones, Cardiac glycosides, steroids/terpenes and quantity yield (Table 1).

Table 1: Qualitative phytochemical screening of the aqueous leaf and seeds extract of *Carica papaya*

Constituents	Leaf	Seeds
Alkaloids	++	+++
Saponins	-	-
Tannins	++	+
Flavonoids	+++	+++
Carbohydrates	++	+
Steroids/Terpenes	++	+
Anthraquinones	-	-
Cardiac glycosides	+	-

Keys: + Present; - Absent

The results of the *in-vitro* nematicidal test indicated that the aqueous seed and leaf extract of *Carica papaya* had nematicidal properties on root-knot nematode juveniles. The test was carried out by the use of different concentrations of the plant extract (0.75, 1.5, 3.0 and 6.0 mg/mL) added to 1 mL of homogenized suspension (50 juveniles) of the nematodes. Each was replicated three

(3) times and kept at room temperature. The mixture was examined at different time intervals of 6, 12, 18 and 24 hours to check for live and dead nematodes. Distilled water with suspension of nematodes without extract was used as control (Table 2 and 3). A comparative analysis of the effect of various concentrations of *Carica papaya* aqueous seed and leave extracts on root-knot nematode juveniles are as shown in Table 4.

Table 2: Effect of various concentration of *Carica papaya* seed aqueous extract on root-knot nematodes second stage juvenile.

Concentration	6		24	LSD
0.75 mg/mL	0.00± 0.00 ^a	3.71± 1.85 ^c	9.26±3.70 ^d	22.22±3.21 ^d
1.5 mg/mL	0.00± 0.00 ^a	38.87± 5.06 ^b	51.85± 4.90 ^c	66.67± 6.41 ^c
3 mg/mL	3.71± 1.85 ^a	50.00± 6.41 ^a	68.15± 2.06 ^b	85.18± 4.89 ^b
6 mg/mL	7.41± 1.85 ^a	57.41± 7.41 ^a	83.32± 3.19 ^a	100.00± 0.00 ^a
Control (Distilled water)	00.00± 0.00 ^a	00.00± 0.00 ^d	00.00± 0.00 ^e	3.71± 1.85 ^e
L.S. D	10.26			

At P≤0.05 there was a significant difference in the nematicidal activity of the crude aqueous extracts of *Carica papaya* leaves on the juvenile of root-knot nematodes. Values are presented as mean±standard error

of means. Ranking was done across the time of exposure and values with lesser figures compared to the LSD is significant, while figures higher than the LSD is not significant.

Table 3: Effect of various concentration of *Carica papaya* aqueous leaves extract on root-knot nematodes juvenile.

Concentration	6	12	18	24	
0.75 mg/mL	0.00± 0.00 ^a	3.71± 1.85 ^d	27.78±9.62 ^d	51.85±8.07 ^c	6.65
1.5 mg/mL	1.85± 1.85 ^a	27.78± 6.41 ^c	61.11± 6.41 ^c	88.89± 6.41 ^b	
3 mg/mL	5.56±3.21 ^a	53.69± 8.08 ^b	77.78± 6.41 ^b	100.00± 0.00 ^a	
6 mg/mL	9.26± 1.85 ^a	83.31± 5.55 ^a	100.00± 0.00 ^a	100.00± 0.00 ^a	
Control (Distilled water)	00.00± 0.00 ^a	00.00± 0.00 ^d	00.00± 0.00 ^e	3.71± 1.85 ^d	
L.S. D	13.30				

At P≤0.05 there was a significant difference in the nematicidal activity of the crude aqueous extracts of *Carica papaya* leaves on the juvenile of root-knot nematodes. Values are presented as mean ± standard error of means. Ranking was done across the time of exposure and values with lesser figures compared to the

LSD is significant, while figures higher than the LSD is not significant. At P≤0.05 there was a significant difference in the nematicidal activity of the crude aqueous extracts of *Carica papaya* leaves on the juvenile of root-knot nematodes. Values are presented as mean ± standard error of means. Ranking was done

across the time of exposure and values with lesser figures compared to the LSD is significant, while figures

higher than the LSD is not significant.

DISCUSSION

The results obtained from this research revealed that the aqueous extract of *Carica papaya* contains biologically active components such as alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids/terpenes, anthraquinones, cardiac glycosides. These bioactive compounds are reported to have nematicidal properties [22]. These bioactive substances may have been responsible for the nematicidal activities of the seed and leaf extracts in this present study. The bioactive substances obtained in this research from seed and leaf extract of *Carica papaya* were found to be present in various quantity. According to Chin *et al.* [23] some of these phytochemicals contributed to the chemotactic, repellent or attractants of nematodes.

However, Rocha *et al.* [24] noted that phytochemical compounds such as serpentine, saponins, phenols, alkaloids, tannins, flavonoids, steroids and cysteine proteinases have anti-helminthic effect against plant parasites and are more active and eco-friendlier, particularly those screened from medicinal and aromatic plants. This could likely be the case with the nematicidal effect of phytochemical screens of plant extracts as observed in the present research.

The results of *in-vitro* experiment showed that the nematode juveniles begin to die at the concentration of 3 mg/ml after 6 hours of exposure under the seed extract, and 1.5 mg/mL under the leaf extract after 6 hours of exposure. The nematicidal activities of the aqueous seeds and leaf extracts of *Carica papaya* increases with concentrations and with time of exposure of nematode juveniles. This agrees to the report of Okechalu *et al.* [12], who reported similar results in the leaf extract of *Ricinus communis* on *Meloidogyne* species.

This study has shown that both the aqueous leaf and seed extracts of *Carica papaya* has nematicidal properties on root-knot nematodes. This agrees with the previous work done by Ayoola and Adeyeye [25] who reported that *Carica papaya* plants produce natural compounds (*Annonaceous acetogenins*) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties.

CONCLUSION

The findings of the present study showed that aqueous leaf and seed extracts of *Carica papaya* both had nematicidal properties on root-knot nematodes. Therefore, the extracts are recommended for the control root-knot nematodes infecting vegetable crops.

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